Brassinosteroids a inter kingdom signaling molecules modulating sterioidogenesis in Polycystic Ovary Syndrome an In Silico study

ABSTRACT

Understanding the influence of ubiquitously present plant hormone brassinosteroids on ovarian sterioidogenesis in polycystic ovarian syndrome (PCOS) is currently of interest. Ovarian tissue sterioidogenesis depends upon the availability of cholesterol besides thecatalytic activity of 17β-dehydrogenases and aromataseis a major regulatory step in ovarian steroidogenicpathway. Ovarian testosterone and estradiol biosynthesis is feedback regulated by GnRH, FSH and LH acting through membrane bound hormone receptors. Brassinosteroids is a polyoxygenated derivative of cholesterol,showing structural similarities with animal oxysterol and available to human through diet, exhibitedantihyperglycemic, anticholesterolemic and antiviral effects. Presentstudy intends to investigatebrassinosteroids againstaromatase, 17β-hydroxysteroid dehydrogenase, androgen and estrogen receptors asa therapeutic target. ADMETproperties of brassinosteroids molecules were evaluated using swiss ADME tool.In Silico molecular docking study were performed via AutoDock version 4.0. Brassinosteroids molecules were exhibits high docking score against the aromatase, 17β-hydroxysteroid dehydrogenase, androgen and estrogen receptors as compared to standard ligand.Dietary intake of brassinosteroids can be potentially down regulatingovarian sterioidogenesis in PCOS.

Keywords: Brassinosteroid, sterioidogenesis, PCOS,molecular docking.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder affecting 8-20% womensworldwide at child bearing age.Being a heterogeneous in nature of PCOS are represented multifaceted symptoms, which predominantly the hyperandrogenism, multiple fluid-filled cysticovarian morphology, anovulation,hirsutism, androgenic alopecia, acne, clitoromegaly and infertility[1].PCOS, in general,altered the metabolic steady statesuch as insulin resistance, increased blood triglyceride, glucose intolerance, obesity, type 2 diabetes, hypertension and cardiovascular diseasetherefore affecting tissue function [2]. Stien and Leventhal in 1935 proposed the PCOS a state associated with amenorrhoea, enlarged ovary, hirsutism and infertility. However,still there is no diagnostic test undeniably determinesPCOS, nevertheless, in 2013 Rotterdam diagnostic criteria proposed and 2018 firm international evidence based guidelines for the assessment and management of PCOS agreed Rotterdam diagnostic criteriabased PCOS diagnosis [3,4].Rotterdam criteria of PCOS subjectat least having two of the three clinical presentations arepolycystic ovarian morphology (PCOM), anovulationand hyperandrogenism.While, based on Rotterdam criteria standard the PCOS subject divided into four phenotypes: (1) hyperandrogenism, anovulation and PCOM, (2) anovulation, PCOMandnon hyperandrogenism (3) anovulation, hyperandrogenism and normal ovaries, (4) PCOM and hyperandrogenism and normal ovulation.Nevertheless, the molecular pathogenesis underlying in PCOS was unclear,however, suggestive of hyperandrogenismcauses high level of estrogentriggered release ofincreased level of luteinizing hormone (LH)and other hand decrease the follicular stimulating hormone (FSH) secretion from the anterior pituitary. The low levels of FSH impede to stimulation, maturation and ovulation and along with increased estrogen level induce endometrial hyperplasia at the end [5]. Hence, FSH secretion can be enhanced by reducing estrogen level with inhibition of aromatase enzyme which converting testosterone to estrogen. Blocking the estrogen receptors are (ERα)another targetfor the FSH secretions enhancement. 17 beta dehydrogenases type 1 (17βHSD) catalyzes the androstenedione to testosterone conversion and inhibiting 17βHSD can be reduced androgen level [6-8]. Now clear that those drug targetson PCOS can be preventing further complications. Currently using drug molecules showed unwanted side effects importantly osteoporosis, impotence and hepatotoxicity [9]. Hence, searching the medicine from plant source is continuously being investigated in PCOS treatment.

Brassinosteroid (BR) is a polyoxygenated sterol comes under class six phyto hormones. In 1979 reported first active form hormone termed brassinolide, followed by 70 hormones in the class identified [10]. BRs ubiquitously present in all the plant and regulating seed germination, growth, development, flowering and present to withstand biotic and abiotic stress effects [11]. However, BRs mimic structural similarity with animal hormones estrogen, androgen and insect ecdysteroid. Brassinosteroids are consumed by human through food and herbal based folk medicine [12]. Assimilation of brassinosteroids and enter into organs through general blood circulation resulted to down regulation of glucose, cholesterol, triglycerides, LDH, proinflamatory cytokines IL-1, TNF-α, COX-2 and up-regulation of glycogen, HDL and transactivation of nuclear receptor in animal cells [13-14].

Conventional pharmacological methods of drug discovery are time-consuming, labour-intensive and expensive. An alternative concept of reverse pharmacology could be a major breakthrough in the field of drug discovery. The basis of reverse pharmacology includes In Silico analogue designing and ligand-receptor interaction and ADMET studies recommendation of chemical nature of molecules [15]. Nevertheless, in the present In Silico study, we intend to investigate the anti-androgenic and anti-estrogenic potential of specific brassinosteroids against aromatase, 17β-hydroxysteroid dehydrogenase type1, androgen receptor and estrogen receptor as an ideal drug target in PCOS.

MATERIAL AND METHODOLOGY

ADMET properties evaluation

Brassinosteroid molecules were evaluatedpharmacokinetic and pharmacodynamic properties of Absorption, Distribution, Metabolism, Execration and Toxicity (ADMET) were determinedusing ADMET predictiononline server (http://admet.scdbb.com)[16].

Ligand preparation

Brassinosteroidand standard compoundsthree-dimensional structures were downloaded from PubChem database (http://pubchem.ncbi.nlm.nih.gov/) as .SDF file format of Brassinolide

(CID: 115196), 28-Homobrassinolide (CID: 11038340), 24-Epibrassinolide (CID: 443055), Castasterone (CID: 133534), 28-Homocastasterone (CID: 5487654), 28-Norbrassinolide (CID: 13845880), 28-Norcastasterone (CID: 13982110), 24-Epicastasterone (CID: 11812633), 3,24-Diepicastasterone (CID: 10961603), 6alpha-Hydroxycastasterone (CID: 15542699), 6-Deoxocastasterone (CID: 13870433), 6-Deoxo-28-norcastasterone (CID: 101682290), Abiraterone (CID: 132971), Androstenedione (CID: 6128), Letrozole (CID: 3902), Anastrozole (CID: 2187), Testosterone (CID: 6013), Flutamide (CID: 3397), Testosterone (CID: 6013), Tamoxifen (CID: 2733526) and Estradiol (CID: 5757) followed by converted into .mol2 file format using Open Babel User Interface software version 2.4.1 and ligands were optimized by means of ligand preparation script in AutoDock ver. 4.0. program. Ligands were prepared for docking as torsion tree, root detection, torsion number were set and saved in .pdbqt file format(Figure 1) [15].

Protein preparation

X-ray crystallographic three-dimensional structures of aromatase (PDB ID: 5JKV), 17β-hydroxysteroid dehydrogenase type1 (PDB ID:1FDS), androgen receptor (PDB ID: 1E3G) and estrogen receptor (PDB ID: 3ERT) were retrieved from RCSB Protein Data Bank (http://www.rcsb.org). Consequently, AutoDockver. 4.0.script employs the removal of water molecules, addition of polar hydrogen atoms, assignment of Kollman charges and conversion of the protein files in .pdb format for further molecular docking study (Figure 2)[15].

Grid box generation

3D structures of selected protein and ligand structure were together to form a grid. Therefore, centroid of the ligand molecule in complex protein structure were chosen to generate grid points X = 60, Y = 60 and Z = 60 axis set for molecular docking. The grid file generated by means of “grid generation panel” in AutoDock software version 4.0 [15].

Molecular docking simulation

Protein-ligand molecular docking performed using AutoDock software version 4.0. For each ligand (chemical structure), 100 docking runs with default parameters were performed by treating protein as rigid and the ligand as flexible. The results were visualized using PyMol (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrodinger, LLC), wherein all the conformations for each of the ligand was found to be within the cavity of protein indicating that the docking run was free from errors. The conformational clusters with lowest binding energy were considered for further analysis [15].

Evaluation of the total binding energy

The AutoDock ver. 4.0. algorithmswere applied to evaluate the total binding energy of ligand against target proteins. Various docked conformations were obtained and one with lowest binding energy towards ligand binding cavity of protein were selected as possible binding conformation and considered for further protein-ligand interaction analysis. The final evaluations of the interactions between the target ligand and amino acid residues of the ligand binding cavity of protein were analyzed using BIOVIA Discovery Studio 2021 script[15].

RESULTS

Brassinosteroids has shown acceptable water solubility (Log S)score with the human intestinal absorption indicative of 90%. While, the Blood-Brain barrier permeabilityshown acceptable range scorethus denotes no ligands molecules have the blood-brain barrier permeability.Also, observed that no brassinosteroids molecules shown CYP450 enzymes inhibition, denoted that ligands metabolized by CYP450 enzyme in liver. Although, evaluation of brassinosteroid molecules bioavailability in blood and tissues noted thatacceptable result indicative of potential bioactive molecule induce cellular effectsand similarly, observed good renal clearance scores. The AMES testshownthere was no mutagenicity property associated withthe brassinosteroids molecules (Table 1 and 2).

The 17βHSD binding interactions with dietary phyto oxysterol brassinosteroids were studied by In Silico docking simulations. Based on docking energies, 17β-Hydroxysteroid dehydrogenase and brassinolide, 28-Homobrassinolide, 24-Epibrassinolide, castasterone, 28-Homocastasterone, 28-Norbrassinolide, 28-Norcastasterone, 24-Epicastasterone, 3,24-Diepicastasterone, 6alpha-Hydroxycastasterone, 6-Deoxocastasterone and 6-Deoxo-28-norcastasterone exhibited binding affinity-6.82, -6.60, -8.78, -9.02, -8.42, -7.75, -8.69, -8.39, -6.78, -9.16, -8.97 and -8.68Kcal/mole respectively as compared to abiraterone and Androstenedionepresented binding affinity -7.68, -7.04Kcal/mol with 17βHSD (Table 3 and 7).

Brassinolide forms hydrogen bond with GLU163, GLU167, ARG266 amino acid residues, while it forms Van der waals interaction with LEU146, THR250, LEU251, LEU260, LEU263 amino acid residues and amino acid residues ARG252, PHE254, LEU267 form Alkyl/π-Alkyl interactions with 17βHSD enzyme. 28-Homobrassinolide forms hydrogen bond with ARG252, ARG266 residues, while it forms Van der waalsinteraction with GLU163, GLY164, THR250, LEU251 amino acid residues and amino acid residues LEU146, PHE254, LEU267 form Alkyl/π-Alkyl interactions with enzyme. 28-Homocastasterone forms hydrogen bond with residues GLY145, while it forms Van der waals interaction with amino acid residues MET147, PHE160, GLU163, GLU167, THR250, LEU251, TYR253 and amino acid residues LEU146, ARG252, PHE254, LEU263, LEU267form Alkyl/π-Alkyl interactions. 28-Norbrassinolide forms hydrogen bond with residues LEU146, ARG266, while it forms Van der waals interaction with amino acid residues GLY145, MET147, GLU163, GLY164, THR250, LEU251, ARG252, PHE254 and amino acid residues PHE160, LEU263, LEU267 form Alkyl/π-Alkyl interactions. 28-Norcastasterone forms hydrogen bond with GLY145 residues, while it forms Van der waals interaction with MET147, PHE160, GLU163, GLU167, THR250, LEU251, LEU260, ARG266 amino acid residues and LEU146, ARG252, PHE254, LEU263 amino acid residues form Alkyl/π-Alkyl interactions. 24-Epicastasterone forms hydrogen bond with GLY145 residues, while it formsVan der waals interaction with MET147, PHE160, GLU163, GLU167, THR250, LEU260, ARG266 amino acid residues and LEU146, ARG252, PHE254, LEU263, LEU267 amino acid residues form Alkyl/π-Alkyl interactions. 3,24-Diepicastasterone forms hydrogen bond with PHE160 residues, while it forms Van der waals interaction with GLY145, GLU163, GLY, 164, GLU167, ARG252, LEU260, ARG264, ARG266 amino acid residues and LEU146, PHE254, LEU263, LEU267 amino acid residues form Alkyl/π-Alkyl interactions. 6alpha-Hydroxycastasterone forms hydrogen bond with GLY145, GLU167 residues, while it forms Van der waals interaction with MET147, PHE160, GLU163, THR220, LEU251, LEU260, ARG266 amino acid residues and LEU146, ARG252, PHE254, LEU263, LEU267 amino acid residues form Alkyl/π-Alkyl interactions. 6-Deoxocastasterone forms hydrogen bond with GLY145, ARG252 residues, while it forms Van der waals interaction with GLU163, GLY164, GLU167, LEU251, ARG266, LEU267 amino acid residues and LEU146, ARG160, PHE254, LEU263 amino acid residues form Alkyl/π-Alkyl interactions. Likewise, 6-Deoxo-28-norcastasterone forms hydrogen bond with GLY145 residues, while it forms Van der waals interaction with MET147, PHE160, GLU163, GLU167, THR250, LEU251, LEU260, ARG266 amino acid residues and LEU146, ARG252, PHE254, LEU263, LEU267 amino acid residues form Alkyl/π-Alkyl interactions with 17βHSD enzyme (Table 3 and Fig 4, 8).

Likewise, the binding studies performed betweenabiraterone and androstenedione and17βHSD enzyme, indicates abiraterone interacting with amino acid residues PHE160via hydrogen bond and with amino acid residues GLY145, GLU163, GLY164, ARG252, PHE254, ARG264, ARG266 via Van der waals interaction and LEU146, LEU260, LEU267 residues via Alkyl/π-Alkyl interactions and amino acid LEU263 via π-sigma. On the other hand, androstenedione interacts with 17βHSD amino acid residues GLY164via hydrogen bond, while GLY145, PHE160, GLU163, GLU167, ARG252, PHE254, ARG266amino acid residues via Van der waals interactions and amino acid residues LEU146, LEU263, LEU267via Alkyl/π-Alkyl interactions with lowest binding affinity with 17βHSD enzyme (Table 7 and fig 3, 8).

The aromatase binding interactions with dietary phyto oxysterol brassinosteroids were studied by In Silico docking simulations. Based on docking energies obtained, aromatase exhibited binding affinity towardsBrassinolide, 28-Homobrassinolide, 24-Epibrassinolide, Castasterone, 28-Homocastasterone, 28-Norbrassinolide, 28-Norcastasterone, 24-Epicastasterone, 3,24-Diepicastasterone, 6alpha-Hydroxycastasterone, 6-Deoxocastasterone and 6-Deoxo-28-norcastasterone is -12.51, -11.54, -12.26, -12.44, -12.36, -12.25, -10.23, -10.99, -11.86, -10.24, -10.53 and -11.10Kcal/molerespectively as compared to letrozole, anastrozole and testosterone presented binding affinity -7.93, -9.6 and -9.85Kcal/mol with aromatase (Table 4 and 7).

In aromatase enzyme interaction, ligand brassinolideinteracted withARG115 and LEU477 residues via hydrogen bond and ILE132, TRP141, ARG145, ALA306, ASP309, THR310, LEU372, VAL373, ARG435, GLY439, SER478 residues via Van der waals interaction and ILE133, PHE134, PHE221, TRP224, VAL370, MET374, CYS437, ALA438residues via Alkyl/π-Alkyl interactions(Table). Similarly, in aromatase-28-Homobrassinolide interaction, ARG115, LEU372, MET374, ALA438, GLY439 amino acid residues form hydrogen bonds with ligand, while PHE221, TRP224, ALA307, THR310, MET311, VAL370, VAL373, ALA443, ILE442, MET446, LEU477, SER478 residues form Van der waals interactions. Ligand 24-Epibrassinolideinteracted with ARG115, LEU477residues via hydrogen bond and ILE132, ILE133, TRP141, ARG145, ALA306, ASP309, THR310, VAL370, LEU372, VAL373, ARG435, CYS437, ALA438, GLY439, SER478 residues viaVan der waals interaction and PHE134, PHE221, TRP224, MET374 residues via Alkyl/π-Alkyl interactions. Ligand Castasteroneinteracted with LEU372, MET374, CYS437residues via hydrogen bond and ILE132, LEU152, PHE221, GLU302, THR310VAL373, GLY439, SER478residues via Van der waals interaction and ILE133, PHE134, PHE148, TRP224, ALA306, MET303, VAL370, ALA438, LEU477residues via Alkyl/π-Alkyl interactions.Ligand 28-Homocastasteroneinteracted with LEU372, MET374, GLY439residues via hydrogen bond and ARG115, LEU151, PHE221, GLU302, MET303, THR310, VAL373, LEU477residues via Van der waals interaction and ILE132, ILE133, PHE134, PHE148, TRP224, ALA306, VAL370, CYS437, ALA438residues via Alkyl/π-Alkyl interactions.Ligand 28-Norbrassinolideinteracted with ARG115, LEU372, LEU477residues via hydrogen bond and ILE132, TRP141, ARG145, PHE221, ALA306, THR310, VAL373, SER478, ARG435, ALA438, GLY439residues via Van der waals interaction and ILE133, PHE134, TRP224, MET374, CYS437residues via Alkyl/π-Alkyl interactions.Ligand 3,24-Diepicastasteroneinteracted with ARG115, LEU477 residues via hydrogen bond and ILE132, TRP141, ARG145, ALA306, ASP310, VAL370, LEU372, VAL373, ARG435, GLY436, SER478residues via Van der waals interaction and ILE133, PHE134, PHE221, TRP224, MET374, CYS437, ALA438residues via Alkyl/π-Alkyl interactions (Table 4 and Fig 5, 9).

Likewise, the binding studies performed between leterozole, anestrozole, testosterone and aromatase enzyme, indicates testosterone interacting with amino acid residues ARG 115, ALA 306, MET 374 via hydrogen bond and amino acid residues PHE134, PHE221, ILE305, ASP309, THR310, ILE133, LEU372, VAL373, LEU477, SER478via Van der waals interaction and TRP224, VAL370residues via Alkyl/π-Alkyl interactions. On the other hand, letrozoleinteracts with aromatase amino acid residues ARGY115, TRP141via hydrogen bond, while ILE132, ILE133, ARG145, LEU152, MET303, ALA306, VAL373, PHE430, GLY431, GLY439amino acid residues via Van der waals interactions and amino acid residues ARG435via Alkyl/π-Alkyl interactions and amino acid residuesCYS437, ALA438 via π-sigma interactions and amino acid residues CYS437 form π-sulfur interaction with lowest binding affinity. Similarly, the anastrozole interacting with amino acid MET311via hydrogen bond and amino acid residuesSER314, THR310, MET364, PRO368, VAL369, PRO429, CYS437, ALA443via Van der waals interaction andPHE430, VAL370via Alkyl/π-Alkyl interactions with aromatase (Table 7 and Fig 3, 9).

In the current In Silico study, androgen receptorwas docked with the dietary phytosterolsbrassinosteroids. The docking scores of Brassinolide, 28-Homobrassinolide, 28-Homocastasterone, 28-Norbrassinolide, 2-Deoxybrassinolide, 28-Norcastasterone, 24-Epicastasterone, 2-Epicastasterone, 3,24-Diepicastasterone, 6-alpha-14-Hydroxycastasterone, 6-Deoxocastasterone, 6-Deoxo-28-norcastasteroneagainst androgen receptor is-5.65, -4.12, -4.42, -5.05, -5.89, -4.25, -4.88, -3.09, - 6.66 and -6.54Kcal/molerespectively as compared to flutamide and testosterone presented binding affinity -0.83 and -1.11 Kcal/mol with androgen receptor (Table 5 and 7).

In androgen receptor, thebrassinolide forms hydrogen bond with amino acid residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions with androgen receptor. 28-Homobrassinolide forms hydrogen bond with residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions with androgen receptor. 28-Homocastasterone forms hydrogen bond with residues while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions. 28-Norbrassinolide forms hydrogen bond with residues while it forms Van der waals interaction with amino acid residues and amino acid residues PHE160, LEU263, LEU267 form Alkyl/π-Alkyl interactions. 28-Norcastasterone forms hydrogen bond with residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions. 24-Epicastasterone forms hydrogen bond with residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions. 3,24-Diepicastasterone forms hydrogen bond with residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions. 6alpha-Hydroxycastasterone forms hydrogen bond with residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions. 6-Deoxocastasterone forms hydrogen bond with residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions. Likewise, 6-Deoxo-28-norcastasterone forms hydrogen bond with residues, while it forms Van der waals interaction with amino acid residues and amino acid residues form Alkyl/π-Alkyl interactions with androgen receptor (Table 5 and Fig 6, 3, 10).

Likewise, the binding studies performed between flutamide, testosterone andandrogen receptor, indicates flutamide interacting with amino acid residues SER740, SER814, GLN867via hydrogen bond and with amino acid residues THR739, MET742, GLY743, LEU744, LEU812, VAL866via Van der waals interaction and LEU811, ILE815, ALA870,ARG871 HIS874, ILE906, PRO913residues via Alkyl/π-Alkyl interactions. On the other hand, testosterone interacts with androgen receptor amino acid residues MET745via hydrogen bond, while GLN711, ALA748, MET780, LEU873, PHE876, THR877amino acid residues via Van der waals interactions and amino acid residues LEU704, LEU707, MET742, VAL746, MET749, PHE764via Alkyl/π-Alkyl interactions with lowest binding affinity as compared to the dietary brassinosteroids (Table 7 and Fig 3, 10).

In the current In Silico study, estrogen receptor was docked with the dietary phytosterols brassinosteroids. The docking scores of Brassinolide, 28-Homobrassinolide, 28-Homocastasterone, 28-Norbrassinolide, 2-Deoxybrassinolide, 28-Norcastasterone, 24-Epicastasterone, 2-Epicastasterone, 3,24-Diepicastasterone, 6-alpha-14-Hydroxycastasterone, 6-Deoxocastasterone, 6-Deoxo-28-norcastasterone against estrogen receptor is-12.96, -9.56, -9.63, -13.33, -10.66, -10.19, -10.98, -12.93, -11.99, -10.66, -12.78 and -11.36 Kcal/mole respectively as compared to tamoxifen and estradiol presented binding affinity and -11.03 and -9.69Kcal/mol with estrogen receptor (Table 6 and 7).

Brassinolide forms hydrogen bond with amino acid residuesASP351 and ASN532, while it forms Van der waals interaction with amino acid residues THR347, LEU384, LEU387, MET388, LEU391, MET421, PHE422, LEU428, VAL534and amino acid residuesLEU346, ALA350, LEU354, TRP383, PHE404, LEU525, MET528, PRO535 form Alkyl/π-Alkyl interactions with estrogen receptor. 28-Homobrassinolide forms hydrogen bond with residuesTHR347, ASP351, GLU353, while it forms Van der waals interaction with amino acid residues LEU349, LEU391, ARG394, PHE404, MET421, ILE424, PHE425, LEU428, ASN532, VAL534and amino acid residues MET343, LEU346, ALA350, LEU354, TRP383, LEU387, LEU525, MET528, PRO535form Alkyl/π-Alkyl interactions with estrogen receptor. Ligand 24-Epibrassinolide interacted with residue ASP351via hydrogen bond and residues MET343, LEU346, THR347, LEU387, LEU391, PHE404, MET421, ILE424, PHE425, LEU428, HIS524, VAL534via Van der waals interaction and ALA350, LEU354, LEU525, VAL533, PRO535residues via Alkyl/π-Alkyl interactions. Ligand Castasterone interacted with residues ASP351, ASN532via hydrogen bond and residues THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, PHE425, LEU428, VAL534via Van der waals interaction and residues LEU346, ALA350, LEU354, PHE404, LEU525, MET528, VAL533, PRO535via Alkyl/π-Alkyl interactions. 28-Homocastasterone forms hydrogen bond with residueTHR347while it forms Van der waals interaction with amino acid residues MET343, GLU353, TRP383, LEU384, MET388, ARG394and LEU346, LEU349, ALA350, LEU387, LEU391, PHE404, LEU428, LEU525amino acid residues form Alkyl/π-Alkyl interactions. 28-Norbrassinolide forms hydrogen bond with ASP351, ASN532residues while it forms Van der waals interaction with amino acid residues MET343, THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, PHE425, LEU428, VAL534and LEU346, ALA350, LEU354, TRP383, PHE404, LEU525, MET528, VAL533, PRO535amino acid residues form Alkyl/π-Alkyl interactions. 28-Norcastasterone forms hydrogen bond with THR347 residue, while it forms Van der waals interaction with amino acid residues ASP351, GLU353, LEU354, LEU384, MET388, LEU391, ARG394, PHE404, PHE425, LEU428, MET528, ASN532, VAL533, VAL534, PRO535and amino acid residues MET343, LEU346, ALA350, TRP383, LEU387, MET421, LEU525form Alkyl/π-Alkyl interactions. 24-Epicastasterone forms hydrogen bond with residues ASP351 and ASN532, while it forms Van der waals interaction with amino acid residues THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, LEU428, VAL534and amino acid residues LEU346, ALA350, LEU354, PHE404, LEU525, MET528, VAL533, PRO535form Alkyl/π-Alkyl interactions. 3,24-Diepicastasterone forms hydrogen bond with residueASP351, while it forms Van der waals interaction with amino acid residues THR347, GLU353, LEU354, LEU391, ARG394, PHE404, PHE425, LEU428, ASN532, VAL533, VAL534, THR347, ARG394, PHE404, MET421, PHE425, LEU428, VAL428, VAL534 and amino acid residues MET343, LEU346, ALA350, TRP383, LEU384, LEU387, MET388, MET421, LEU525, PRO535form Alkyl/π-Alkyl interactions. 6α-Hydroxycastasterone forms hydrogen bond with ASN532 residue, while it forms Van der waals interaction with LEU346, THR347, LEU349, ASP351, GLU353, LEU354, MET421, ILE424, PHE425, LEU428, GLY521, VAL534amino acid residues and amino acid residues ALA350, TRP383, LEU384, LEU387, MET388, LEU391, PHE404, VAL533, PRO535form Alkyl/π-Alkyl interactions. 6-Deoxocastasterone forms Van der waals interaction with MET343, THR347, ASP351, GLU353, TRP383, LEU384, MET388, ARG394, MET421, PHE425, ASN532, PRO535amino acid residues and LEU346, LEU349, ALA350, LEU387, LEU391, PHE404, LEU525, VAL533amino acid residues form Alkyl/π-Alkyl interactions. Likewise, 6-Deoxo-28-norcastasterone forms LEU346, LEU347, GLU353, TRP383, MET388, ARG394, PHE404, PRO535Van der waals interaction with amino acid residues and amino acid residues MET343, ALA350, LEU384, LEU387, LEU391, MET421, ILE424, PHE425, LEU428, LEU525, VAL533form Alkyl/π-Alkyl interactions with estrogen receptor (Table 6 and Fig 7, 3, 11).

Likewise, the binding studies performed between thetamoxifen, estradiol andestrogen receptor, indicates interacting with amino acid residues ASP351, VAL534via hydrogen bond and with amino acid residues GLU353, LEU354, TRP383, LEU384, MET388, MET421, ILE424, PHE425, GLY521, HIS524, VAL533, PRO535via Van der waals interaction and LEU346, LEU349, ALA350, LEU387, PHE404, LEU525residues via Alkyl/π-Alkyl interactions and amino acid residueLEU525 form π-sigma interaction. On the other hand, estradiol interacts with estrogen receptor amino acid residues GLU353, ARG394, GLY521via hydrogen bond, while amino acid residuesLEU346, LEU349, MET421, LEU428, HIS524 via Van der waals interactions and amino acid residues ALA350, LEU384, LEU387, MET388, LEU391, ILE424, LEU525via Alkyl/π-Alkyl interactions and amino acid residue PHE404form π-sigma interaction with lowest binding affinity as compared to the dietary brassinosteroids (Table 7 and Fig 3, 11).

DISCUSSION

Understanding on enzyme and cellular receptors functions in ovarian steroidogenic pathways provides several occurrences of feedback regulation. Proof of ovarian steroidogenic pathway modulator through In Silico methods have accepted exogenous molecules act as active site modifiers of specific enzymatic function in steroidogenic metabolic pathways, indicating rate limiting regulatory phenomena in cells.The important enzymatic regulatory step in the steroidogenic biosynthetic pathway leading to the synthesisof testosterone, estrogen in ovarian cells is that involving the transformation ofcholesterolto pregnenolone(enzyme P450scc)intoandrostenedione to testosterone (enzyme 17βHSD) and testosterone to estradiol(aromatase) in the theca and granulosa cells. Further, androgen and estrogenbiosynthesis and homeostasis are under the regulation of GnRH, LH and FSH signaling mediators [17].

Human 7β-hydroxysteroid dehydrogenase type 1 is comes under the steroid dehydrogenase reductase family. 17βHSD catalyzes reduction of androstenedione to testosterone, in the presence of NADPH as a cofactor in the ovarian granulosa cell [18]. The hyperandrogenism a main hallmark in PCOS resulted to anovulation, polycystic ovarian morphology, which can be over expression of 17βHSD enzyme. Hence 17βHSD enzyme inhibition can be ideal therapeutic management in PCOS individual [19]. In the present In Silico study observed thatvaluable insights between brassinosteroids ligands with 17βHSD a key enzymeinvolved in ovarian sterioidogenesis pathway. The brassinosteroids compounds 6α-Hydroxycastasterone, castasterone, 6-Deoxocastasterone, 24-Epibrassinolide, 6-Deoxo-28-norcastasteroneshown high binding avidity towards 17βHSD enzyme compared to the reference compounds abiraterone (-7.68kcal/mol) and androstenedione (-7.04kcal/mol) highlights their promising inhibitors for the 17βHSD enzyme (Table 3 and 7).

Individual with PCOS causes the oligomenorrhoea, amenorrhoea and anovulation resulted by higher level of estrogen in PCOS, that affecting normal ovarian physiology. The aromatase enzyme inhibitoris make ovulation happen in PCOS.The aromatase enzyme comes under the cytochrome p450 family, secreted by granulosa cell of ovary and catalyzes the conversion of testosterone to estrogen irreversibly. This catalytic reaction is the final and rate limiting step in the ovarian estrogen synthesis [20, 21].In the present In Silico study observed that brassinolide, castasterone, 28-Homocastasterone, 24-Epibrassinolide, 28-Norbrassinolide, 3,24-Diepicastasterone, 28-Homobrassinolide, 6-Deoxo-28-norcastasterone shown highest binding affinities towards aromatase enzyme compared to reference compounds testosterone (-9.85kcal/mol), anastrozole (-9.60kcal/mol) and letrozole (-7.93kcal/mol) thathighlights their promising inhibitors for the aromatase enzyme(Table 4 and 7).However, daily intake of brassinosteroids may be down regulate estrogen level resulted topreventing the PCOS effects on ovulation.

Androgen receptor mediated signaling mechanism in PCOS resulted byaltered phenotype traitsthat down regulation of follicles development leading to multiple small cysts in ovary caused the anovulation,hirsutism, acne andalopecia in peripheral tissue[22].Therefore, inhibition of androgen with androgen receptor interaction is idyllic phenomenon to managing PCOS complications.Although in the present workthe brassinosteroids compounds 6-Deoxocastasterone,6-Deoxo-28-norcastasterone, 28-Norcastasterone, brassinolide, 28-Norbrassinolide, 3,24-Diepicastasterone, 28-Homocastasterone, 28-Homobrassinolide shown highest binding avidity towards androgen receptor compared to reference compoundstestosterone (-1.11kcal/mol) and flutamide (-0.83kcal/mol)(Table 5 and 7) to inhibition of androgen receptor potentially.However, the brassinosteroids may be down regulate androgen receptor impact on the PCOS individual and prevent complications.

Estrogen receptorcan be localized in cell membrane, cytoplasm and nuclease. The estrogen interact with estrogen receptor resulted to genomic or non-genomic effects such as transcriptional and cell divisioneffects on target tissues. In PCOS estrogen receptor inducing estrogen synthesis that causes increased level of estrogen resulted to anovulation. Therefore, targeting estrogen receptor in PCOS individual is idyllic phenomenon [23, 24]. Therefore, in the present investigationcarried out estrogen receptor inhibition effects of the brassinosteroids compounds castasterone, brassinolide, 24-Epicastasterone, 6-Deoxocastasterone, 3,24-Diepicastasterone, 6-Deoxo-28-norcastasteroneshown highest binding affinities towards estrogen receptor compared to reference compounds tamoxifen (-11.03kcal/mol) and estradiol (-9.69kcal/mol)(Table 6 and 7)highlights their promising inhibitors of the estrogen receptor in PCOS.

Drug molecules with poor pharmacokinetic and pharmacodynamic properties can have adverse impact on human biological system such as alter organ function, immunological reaction, and dermatological issues. However, present investigation the brassinosteroids ADMET properties evaluated. 12 brassinosteroids was evaluated for the water solubility, human intestinal absorption, blood brain barrier permeability, CYTp450 isoforms inhibitor or substrate, bioavailability, renal clearance and mutagenic effect using the ADMET online tool. Brassinosteroids has shown the acceptable ADMET results in order intestinal absorption into circulatory system to reach target tissues followed by bio-physiological effects and metabolized in liver than excreted in urine.

Ovarian sterioidogenesis a rate limiting steps in testosterone and estrogen biosynthesis had been recognized as catalytic regulation of 17βHSD and aromatase enzyme, the effects of brassinosteroids on enzyme catalytic function was studied by In Silico analysis of interaction between 17βHSD and aromatase enzyme, androgen and estrogen receptor with brassinosteroid ligands. The significant binding avidity between the testosterone, estrogen and brassinosteroid on enzyme catalytic side, suggestive a modulatory effect by brassinosteroids on ovarian sterioidogenesisin cell.In the present investigation highlights the effects of brassinosteroids in its capability to down regulatingtestosterone and estrogen levels and inhibition of androgen and estrogen receptors in PCOS. Further,an In Vivo study is needed to understanding the possible influences contributing brassinosteroids to ovarian sterioidogenesis in normal and PCOS subjects.

CONCLUSION

Present In Silico study identified 6α-Hydroxycastasterone, castasterone, 28-Homocastasterone,6-Deoxocastasterone, 24-Epibrassinolide, 6-Deoxo-28-norcastasterone, brassinolide, 28-Norbrassinolide, 3,24-Diepicastasterone, and 28-Homobrassinolideas potential modulator of ovarian sterioidogenesis through 17βHSD, aromatase, androgen and estrogen receptors inhibition. Among them 6-Deoxo-28-norcastasterone, castasterone, 24-Epibrassinolide, 6-Deoxocastasterone, 3,24-Diepicastasterone, 28-Norbrassinolide and brassinolideare exhibited superior putative ovarian sterioidogenesis inhibitor. These results suggestive a novel phyto molecule based managing PCOS complications.Remarkably, brassinosteroids shown better binding avidity with 17βHSD, aromatase, androgenand estrogen receptors than conventional drug molecules. The outcome of our In Silico data may be the basis for In Vivo and In Vitro studies against PCOS with phyto molecules brassinosteroids.

Conflicts of interest: Declare that there are no conflicts of interest, whatsoever, among themselves.

Animal and human ethics clearance: Not applicable

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Tables and Figures

Table 1. Evaluation of drug-likeness of brassinosteroids molecules using Lipinski rule of five

Compounds	Molecular Weight (Da)	H-bond donors	H-bond acceptors	LogP Values
Brassinolide	480.686	4	6	3.390
28-Homobrassinolide	494.713	4	6	3.780
24-Epibrassinolide	480.686	4	6	3.390
Castasterone	464.687	4	5	3.806
28-Homocastasterone	478.714	4	5	4.190
28-Norbrassinolide	466.659	4	6	3.140

28-Norcastasterone	450.660	4	5	3.560
24-Epicastasterone	464.687	4	5	3.806
3,24-Diepicastasterone	464.687	4	5	3.806
6α-Hydroxycastasterone	466.703	5	5	3.598
6-Deoxocastasterone	450.704	4	4	4.627
6-Deoxo-28-norcastasterone	436.677	4	4	4.381

Table 2.ADME properties of Brassinosteroids compounds

ADME Properties	1	2	3	4	5	6	7	8	9	10
Absorption Properties										
Caco-2 Permeability Optimal: higher than -5.15 Log unit or -4.70 or -4.80	-4.791 cm/s	-4.827 cm/s	-4.791cm/s	-4.791cm/s	-4.793 cm/s	-4.846 cm/s	-4.842	-4.793	-4.793	-4.836
Human Intestinal Absorption ≥30%: HIA+; <30%: HIA-	0.611	0.599	0.611	0.611	0.689	0.592	0.684	0.689	0.689	0.685
P-glycoprotein Substrate	0.101	0.166	0.101	0.101	0.08	0.151	0.123	0.080	0.080	0.167
P- glycoprotein Inhibitor	0.384	0.619	0.384	0.384	0.36	0.622	0.603	0.360	0.360	0.330
Distribution Properties										
Plasma Protein Binding90%	84.529	84.502	84.529	84.529	83.784	83.931	83.935	83.784	83.784	82.012
Blood brain barrier (BBB) BB ratio >=0.1: BBB+; BB ratio <0.1: BBB-	0.406	0.407	0.418	0.418	0.808	0.388	0.764	0.808	0.808	0.818
Volume Distribution 0.04-20 L/kg	-0.468	-0.523	-0.468	-0.468	-0.297	-0.411	-0.247	-0.297	-0.297	-0.243
Metabolism Properties										
P450 CYP1A2-inhibitor	0.039	0.057	0.039	0.039	0.028	0.050	0.039	0.028	0.028	0.026
P450 CYP1A2-substrate	0.318	0.370	0.318	0.318	0.330	0.362	0.370	0.330	0.330	0.293
P450 CYP3A4-inhibitor	0.203	0.319	0.203	0.203	0.109	0.355	0.216	0.109	0.109	0.133
P450 CYP3A4-substrate	0.667	0.683	0.667	0.667	0.687	0.658	0.688	0.687	0.687	0.587
P450 CYP2C9-inhibitor	0.195	0.289	0.195	0.195	0.116	0.234	0.151	0.116	0.116	0.137
P450 CYP2C9-substrate	0.185	0.214	0.185	0.185	0.194	0.194	0.193	0.194	0.194	0.217
P450 CYP2C19-inhibitor	0.095	0.108	0.095	0.095	0.057	0.106	0.059	0.057	0.057	0.107
P450 CYP2C19substrate	0.552	0.536	0.552	0.552	0.470	0.567	0.481	0.470	0.470	0.465
P450 CYP2D6-inhibitor	0.267	0.289	0.267	0.095	0.245	0.279	0.261	0.245	0.245	0.301
P450 CYP2D6-substrate	0.222	0.207	0.222	0.222	0.255	0.240	0.270	0.255	0.255	0.337
Excretion Properties										
T 1/2 (Half Life Time) >8h: high, 3h<Cl< 8h: moderate,<3h: low	1.392	1.447	1.392	1.392	1.551	1.347	1.543	1.551	1.551	1.565
ClearancemL/min/kg >15 mL/min/kg: high; 5mL/min/kg<Cl< 15mL/min/kg: moderate; <5 mL/min/kg: low	1.382	1.32	1.382	1.382	1.385	1.436	1.447	1.385	1.385	1.355
Toxicity properties										
hERG Blockers Category 0: Non-blockers Category 1: Blockers	-0.353	-0.361	-0.353	0.353	0.401	0.345	0.387	0.401	0.401	0.417
AMES test Category 0: Ames test -ve Category 1: Ames test +ve	0.186	0.182	0.186	0.186	0.224	0.212	0.288	0.224	0.224	0.302
Drug Induced Liver Injury Category 0: DILI -veCategory 1: DILI +ve	-0.360	-0.242	-0.360	-0.360	0.226	0.244	0.112	0.226	0.226	0.124
PhysicochemicalProperties										

Log S (Solubility) Optimal: higher than -4 log mol/L	-4.836	-5.083	-4.836	-4.836	-5.19	-4.388	-5.012	-5.19	-5.19	-5.056
Distribution Coefficient 1 to 3: Solubility moderate, Permeability moderate, Metabolism low.	3.318	3.489	3.318	3.318	3.521	3.294	3.565	3.521	3.521	3.516
LogP (LogP <0: poor lipid bilayer permeability. LogP >3: poor aqueous.	3.39	3.78	3.39	3.39	3.806	3.144	3.56	3.806	3.806	3.598

Compounds:1, Brassinolide, 2. 28-Homobrassinolide, 3. 24-Epibrassinolide, 4. Castasterone, 5. 28-Homocastasterone, 6.28-Norbrassinolide, 7. 28-Norcastasterone, 8.24-Epicastasterone, 9.3,24-Diepicastasterone, 10. 6α-Hydroxycastasterone.

Table.3. Brassinosteroids and 17β-Hydroxysteroid-dehydrogenase enzyme interaction

17βHydroxysteroid dehydrogenase. (1FDS)	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl
Brassinolide	-6.82	LEU146, THR250, LEU251, LEU260, LEU263.	GLU163, GLU167, ARG266.	ARG252, PHE254, LEU267.
28-Homobrassinolide	-6.60	GLU163, GLY164, THR250, LEU251.	ARG252, ARG266.	LEU146, PHE254, LEU267.
24-Epibrassinolide	-8.78	GLU163, GLY164, GLU167, LEU251, ARG252, PHE254, ARG266, LEU267.	GLY145	LEU146, PHE160, LEU263.
Castasterone	-9.02	MET147, PHE160, GLU163, GLU167, THR250, LEU251, ARG266.	GLY145	LEU146, ARG252, PHE254, LEU263, LEU267.
28-Homocastasterone	-8.42	MET147, PHE160, GLU163, GLU167, THR250, LEU251, TYR253.	GLY145	LEU146, ARG252, PHE254, LEU263, LEU267.
28-Norbrassinolide	-7.75	GLY145, MET147, GLU163, GLY164, THR250, LEU251, ARG252, PHE254.	LEU146, ARG266.	PHE160, LEU263, LEU267.
28-Norcastasterone	-8.69	MET147, PHE160, GLU163, GLU167, THR250, LEU251, LEU260, ARG266.	GLY145	LEU146, ARG252, PHE254, LEU263.
24-Epicastasterone	-8.39	MET147, PHE160, GLU163, GLU167, THR250, LEU260, ARG266.	GLY145	LEU146, ARG252, PHE254, LEU263, LEU267.
3,24-Diepicastasterone	-6.78	GLY145, GLU163, GLY, 164, GLU167, ARG252, LEU260, ARG264, ARG266.	PHE160	LEU146, PHE254, LEU263, LEU267.
6αHydroxycastasterone	-9.16	MET147, PHE160, GLU163, THR220, LEU251, LEU260, ARG266.	GLY145, GLU167.	LEU146, ARG252, PHE254, LEU263, LEU267.
6-Deoxocastasterone	-8.97	GLU163, GLY164, GLU167, LEU251, ARG266, LEU267.	GLY145, ARG252.	LEU146, ARG160, PHE254, LEU263.
6-Deoxo-28-norcastasterone	-8.68	MET147, PHE160, GLU163, GLU167, THR250, LEU251, LEU260, ARG266.	GLY145.	LEU146, ARG252, PHE254, LEU263, LEU267.

Table.4. Brassinosteroids and aromatase enzyme interaction

Aromatase (5JKV)	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl
Brassinolide	-12.51	ILE132, TRP141, ARG145, ALA306, ASP309, THR310, LEU372, VAL373, ARG435, GLY439, SER478.	ARG115, LEU477	ILE133, PHE134, PHE221, TRP224, VAL370, MET374, CYS437, ALA438.
28-Homobrassinolide	-11.54	PHE221, TRP224, ALA307, THR310, MET311, VAL370, VAL373, ALA443, ILE442, MET446, LEU477, SER478.	ARG115, LEU372, MET374, ALA438, GLY439	-
24-Epibrassinolide	-12.26	ILE132, ILE133, TRP141, ARG145, ALA306, ASP309, THR310, VAL370, LEU372, VAL373, ARG435, CYS437, ALA438, GLY439, SER478.	ARG115, LEU477	PHE134, PHE221, TRP224, MET374.

Castasterone	-12.44	ILE132, LEU152, PHE221, GLU302, THR310VAL373, GLY439, SER478.	LEU372, MET374, CYS437.	ILE133, PHE134, PHE148, TRP224, ALA306, MET303, VAL370, ALA438, LEU477.
28-Homocastasterone	-12.36	ARG115, LEU151, PHE221, GLU302, MET303, THR310, VAL373, LEU477.	LEU372, MET374, GLY439.	ILE132, ILE133, PHE134, PHE148, TRP224, ALA306, VAL370, CYS437, ALA438.
28-Norbrassinolide	-12.25	ILE132, TRP141, ARG145, PHE221, ALA306, THR310, VAL373, SER478, ARG435, ALA438, GLY439.	ARG115, LEU372, LEU477	ILE133, PHE134, TRP224, MET374, CYS437.
28-Norcastasterone	-10.23	ARG115, ILE132, PHE134, LEU152, PHE221, ALA307, THR310, MET443, MET446, LEU477, SER478.	GLY439	ILE133, PHE148, TRP224, MET303, ALA306, VAL370, ALA438.
24-Epicastasterone	-10.99	ARG115, ILE132, PHE134, ARG145, ASP309, LEU372, MET374, ALA443, SER478.	CYS437, ALA438, GLY439, LEU477.	ILE133, PHE221, TRP224, MET303, ALA306, VAL370, ALA438.
3,24-Diepicastasterone	-11.86	ILE132, TRP141, ARG145, ALA306, ASP310, VAL370, LEU372, VAL373, ARG435, GLY436, SER478.	ARG115, LEU477.	ILE133, PHE134, PHE221, TRP224, MET374, CYS437, ALA438.
6α-Hydroxycastasterone	-10.24	ARG115, ILE132, PHE221, ALA307, THR310, MET311, VAL373, CYS437,GLY439, LEU477, SER478.	-	ILE133, PHE148, LEU152, TRP224, MET303, ALA306, VAL370, ALA438, ILE442, ALA443, MET446.
6-Deoxocastasterone	-10.53	ARG115, ILE133, PHE221, MET311, MET364, MET374, PRO429, MET447.	THR310, LEU372.	PHE134, TRP224, VAL370, VAL373, PHE430, CYS437, ALA443, LEU477.
6-Deoxo-28-norcastasterone	-11.10	ARG115, TRP224, ASP309, THR310, VAL369, VAL370, LEU372, MET374, GLY439, SER478.	CYS437, ALA438, LEU477.	ILE133, PHE134, PHE221, ALA306, ALA438.

Table 5. Brassinosteroids and Androgen receptoramino acid residues interaction

Androgen receptor (1E3G)	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl
Brassinolide	-5.65	LEU701, ASN705, LEU707, GLY708, TRP741, MET745, ARG752, PHE764, LEU768, GLN783, MET787, THR877, LEU880, PHE891, ILE899.	VAL746	LEU704, MET742, MET780, LEU873, PHE876, MET895
28-Homobrassinolide	-4.12	LEU701, ASN705, LEU707, GLY708, TRP741, MET745, MET749, ARG752, GLN783, PHE764, MET787, LEU880, PHE891, ILE899.	VAL746, THR877	LEU704, MET742, MET780, PHE876, MET895.
28-Homocastasterone	-4.42	LEU701, LEU707, GLY708, GLN711, TRP741, MET749, PHE764, MET787, THR877, ILE899	ASN705, VAL746, ARG752.	LEU704, MET742, MET745, MET780, LEU873, PHE876, MET895.
28-Norbrassinolide	-5.05	LEU701, LEU704, LEU707, GLY708, MET745, MET749, ARG752, LEU768, MET787, THR877, LEU880, PHE891.	ASN705, VAL746.	MET742, MET780, LEU873, PHE876, MET895.
28-Norcastasterone	-5.89	GLU681, GLY683, VAL685, HIS714, LEU744, MET745, TRP751, THR755, ASN756, PRO766, LYS808.	GLN711	PRO682, VAL684, VAL715, ALA748, ARG752
24-Epicastasterone	-4.25	GLY708, TRP741, LEU880, VAL889, PHE891, MET895.	LEU701, ASN705, GLN711, PHE764, THR877.	LEU704, LEU707, MET742, MET745, VAL746, MET749, MET780, MET787, LEU823, PHE876.
3,24-Diepicastasterone	-4.88	LEU701, LEU707, GLY708, TRP741, ALA748, MET749, PHE764, MET787, THR877, LEU880, PHE891.	ASN705, GLN711, VAL746, ARG752.	LEU704, MET742, MET745, MET780, LEU873, PHE876, MET895.
6α Hydroxycastasterone	-3.09	LEU701, LEU707, GLY708, GLN711, TRP741, ARG752, THR877, LEU880, PHR891, ILE899.	ASN705	LEU705, MET742, MET745, VAL746, MET749, PHE764, MET780, MET787, LEU873, PHE876, MET895.
6-Deoxocastasterone	- 6.66	LEU701, ASN705, LEU707, TRP741, MET742, ALA748, GLN783, THR877, LEU880, PHE891, ILE899.	GLN711, ARG752, PHE762.	LEU704, MET745, VAL746, MET749, MET780, MET787, LEU873, MET780, PHE876, MET895.
6-Deoxo-28-	-6.54	LEU701, LEU704, ASN705, LEU707, GLY708, TRP741, ARG752, GLN783, THR877,	-	MET742, MET745, VAL746, MET749, PHE764, MET780, MET787,LEU873,

norcastasterone		LEU880, PHE891.		PHE876,MET895.

Table 6. Brassinosteroids and Estrogen receptor amino acid residues interaction

Estrogen receptor (3ERT)	**G-Score kcal/mol**	**Van der waals**	**Hydrogen bond**	**Alkyl/π-Alkyl**
Brassinolide	-12.96	THR347, LEU384, LEU387, MET388, LEU391, MET421, PHE422, LEU428, VAL534.	ASP351, ASN532.	LEU346, ALA350, LEU354, TRP383, PHE404, LEU525, MET528, PRO535.
28-Homobrassinolide	-9.56	LEU349, LEU391, ARG394, PHE404, MET421, ILE424, PHE425, LEU428, ASN532, VAL534.	THR347, ASP351, GLU353.	MET343, LEU346, ALA350, LEU354, TRP383, LEU387, LEU525, MET528, PRO535.
24-Epibrassinolide	-9.63	MET343, LEU346, THR347, LEU387, LEU391, PHE404, MET421, ILE424, PHE425, LEU428, HIS524, VAL534.	ASP351	ALA350, LEU354, LEU525, VAL533, PRO535.
Castasterone	-13.33	THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, PHE425, LEU428, VAL534.	ASP351, ASN532.	LEU346, ALA350, LEU354, PHE404, LEU525, MET528, VAL533, PRO535.
28-Homocastasterone	-10.66	MET343, GLU353, TRP383, LEU384, MET388, ARG394.	THR347	LEU346, LEU349, ALA350, LEU387, LEU391, PHE404, LEU428, LEU525.
28-Norbrassinolide	-10.19	MET343, THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, PHE425, LEU428, VAL534.	ASP351, ASN532.	LEU346, ALA350, LEU354, TRP383, PHE404, LEU525, MET528, VAL533, PRO535.
28-Norcastasterone	-10.98	ASP351, GLU353, LEU354, LEU384, MET388, LEU391, ARG394, PHE404, PHE425, LEU428, MET528, ASN532, VAL533, VAL534, PRO535.	THR347.	MET343, LEU346, ALA350, TRP383, LEU387, MET421, LEU525.
24-Epicastasterone	-12.93	THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, LEU428, VAL534.	ASP351, ASN532.	LEU346, ALA350, LEU354, PHE404, LEU525, MET528, VAL533, PRO535.
3,24-Diepicastasterone	-11.99	THR347, GLU353, LEU354, LEU391, ARG394, PHE404, PHE425, LEU428, ASN532, VAL533, VAL534, THR347, ARG394, PHE404, MET421, PHE425, LEU428, VAL428, VAL534.	ASP351.	MET343, LEU346, ALA350, TRP383, LEU384, LEU387, MET388, MET421, LEU525, PRO535.
6αHydroxycastasterone	-10.66	LEU346, THR347, LEU349, ASP351, GLU353, LEU354, MET421, ILE424, PHE425, LEU428, GLY521, VAL534.	ASN532	ALA350, TRP383, LEU384, LEU387, MET388, LEU391, PHE404, VAL533, PRO535.
6-Deoxocastasterone	-12.78	MET343, THR347, ASP351, GLU353, TRP383, LEU384, MET388, ARG394, MET421, PHE425, ASN532, PRO535.	-	LEU346, LEU349, ALA350, LEU387, LEU391, PHE404, LEU525, VAL533.
6-Deoxo-28-norcastasterone	-11.36	LEU346, LEU347, GLU353, TRP383, MET388, ARG394, PHE404, PRO535.	-	MET343, ALA350, LEU384, LEU387, LEU391, MET421, ILE424, PHE425, LEU428, LEU525, VAL533.

Table 7. Standard ligands and sterioidogenesis proteins amino acid residues interaction

Proteins	**Compounds**	**G-Score kcal/mol**	**Van der waals**	**Hydrogen bond**	**Alkyl/π-Alkyl**	**π-sigma**
17βhydroxysteroid dehydrogenase (1FDS)	**Abiraterone**	-7.68	GLY145, GLU163, GLY164, ARG252, PHE254, ARG264, ARG266.	PHE160	LEU146, LEU260, LEU267.	LEU263
	Androstenedione	-7.04	GLY145, PHE160, GLU163, GLU167, ARG252, PHE254, ARG266.	GLY164	LEU146, LEU263, LEU267.	-

Aromatase (5JKV)	**Letrozole**	-7.93	ILE132, ILE133, ARG145, LEU152, MET303, ALA306, VAL373, PHE430, GLY431, GLY439	ARGY115, TRP141	ARG435	CYS437
	Anastrozole	-9.6	SER314, THR310, MET364, PRO368, VAL369, PRO429, CYS437, ALA443.	MET311	PHE430, VAL370	-
	Testosterone	-9.85	PHE134, PHE221, ILE305, ASP309, THR310, ILE133, LEU372, VAL373, LEU477, SER478.	ARG 115, ALA 306, MET 374.	TRP224, VAL370	-
Androgen receptor (1E3G)	**Flutamide**	-0.83	THR739, MET742, GLY743, LEU744, LEU812, VAL866.	SER740, SER814, GLN867.	LEU811, ILE815, ALA870,ARG871 HIS874, ILE906, PRO913.	-
	Testosterone	-1.11	GLN711, ALA748, MET780, LEU873, PHE876, THR877.	MET745	LEU704, LEU707, MET742, VAL746, MET749, PHE764.	-
Estrogen receptor (3ERT)	**Tamoxifen**	-11.03	GLU353, LEU354, TRP383, LEU384, MET388, MET421, ILE424, PHE425, GLY521, HIS524, VAL533, PRO535.	ASP351, VAL534	LEU346, LEU349, ALA350, LEU387, PHE404, LEU525	LEU525
	Estradiol	-9.69	LEU346, LEU349, MET421, LEU428, HIS524.	GLU353, ARG394, GLY521.	ALA350, LEU384, LEU387, MET388, LEU391, ILE424, LEU525.	PHE404

Figures

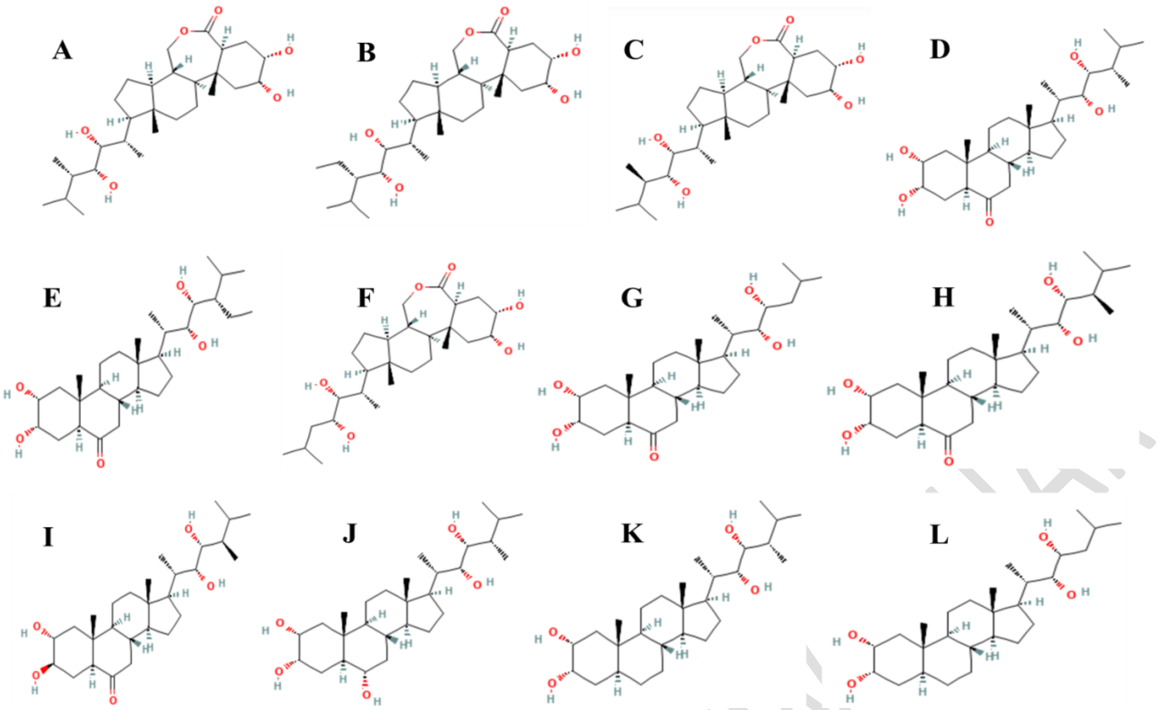


Figure 1. Shows 2D structure of ligands A. Brassinolide, B. 28-Homobrassinolide, C. 24-Epibrassinolide, D. Castasterone, E. 28-Homocastasterone, F. 28-Norbrassinolide, G. 28-Norcastasterone, H. 24-Epicastasterone, I. 3,24-Diepicastasterone, J. 6αHydroxycastasterone, K. 6-Deoxocastasterone, L. 6-Deoxo-28-norcastasterone.

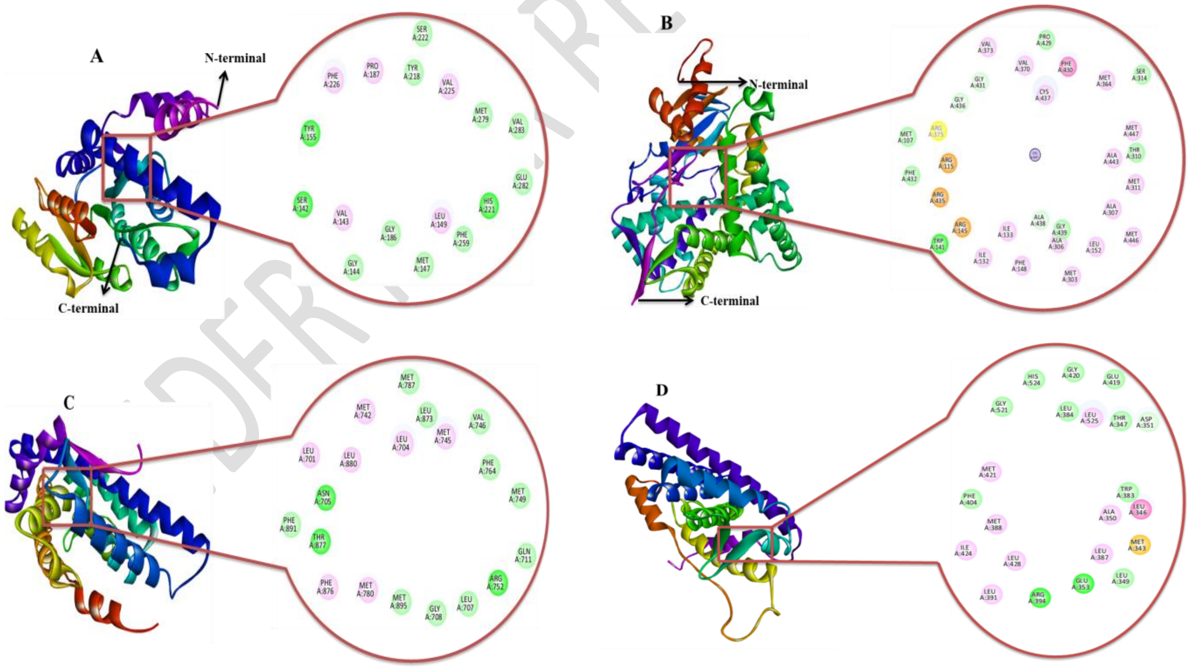


Figure 2. Shows the ligand binding side and the amino acid residues interacting ligand, A. 17βHSD enzyme, B. Aromatase enzyme, C. Androgen receptor, D. Estrogen receptor.

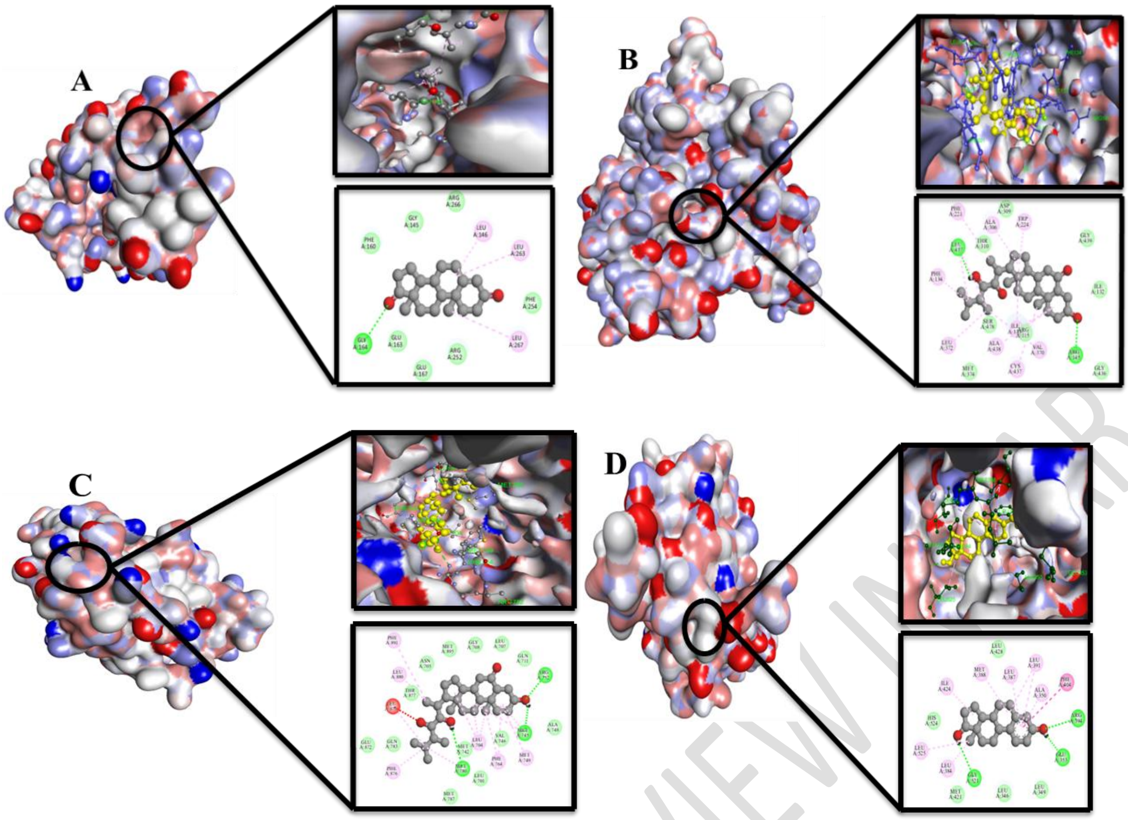


Figure 3. Shows the 2D and 3D interaction of A.17βHSD enzyme and Androstenedione, B. Aromatase enzyme and testosterone, C. Androgen receptor and testosterone, D. Estrogen receptor and estradiol.

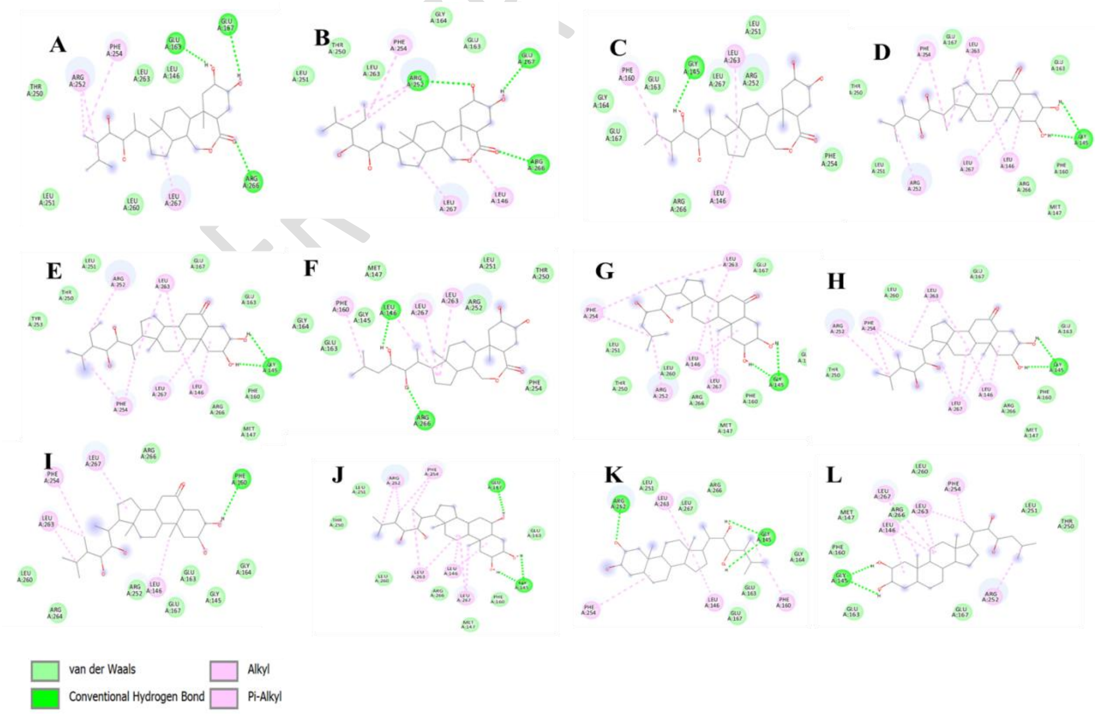


Figure 4. Shows A. Brassinolide, B. 28-Homobrassinolide, C. 24-Epibrassinolide, D. Castasterone, E. 28-Homocastasterone, F. 28-Norbrassinolide, G. 28-Norcastasterone, H. 24-Epicastasterone, I. 3,24-Diepicastasterone, J. 6αHydroxycastasterone, K. 6-Deoxocastasterone, L. 6-Deoxo-28-norcastasterone.

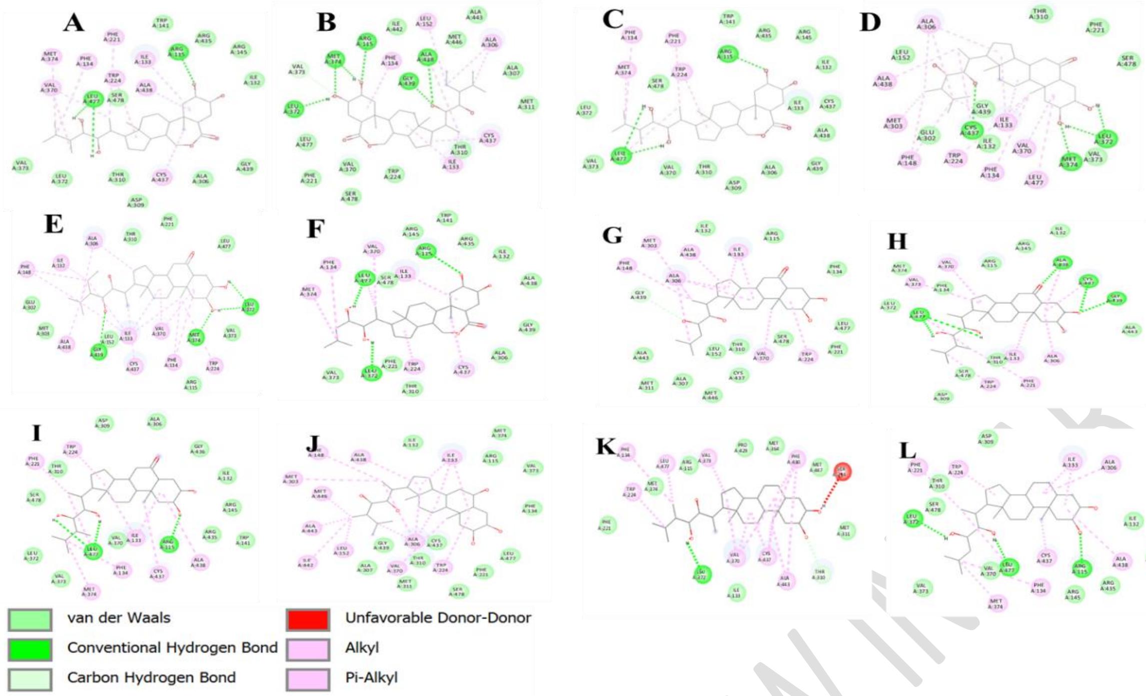


Figure 5. Shows A. Brassinolide, B. 28-Homobrassinolide, C. 24-Epibrassinolide, D. Castasterone, E. 28-Homocastasterone, F. 28-Norbrassinolide, G. 28-Norcastasterone, H. 24-Epicastasterone, I. 3,24-Diepicastasterone, J. 6αHydroxycastasterone, K. 6-Deoxocastasterone, L. 6-Deoxo-28-norcastasterone.

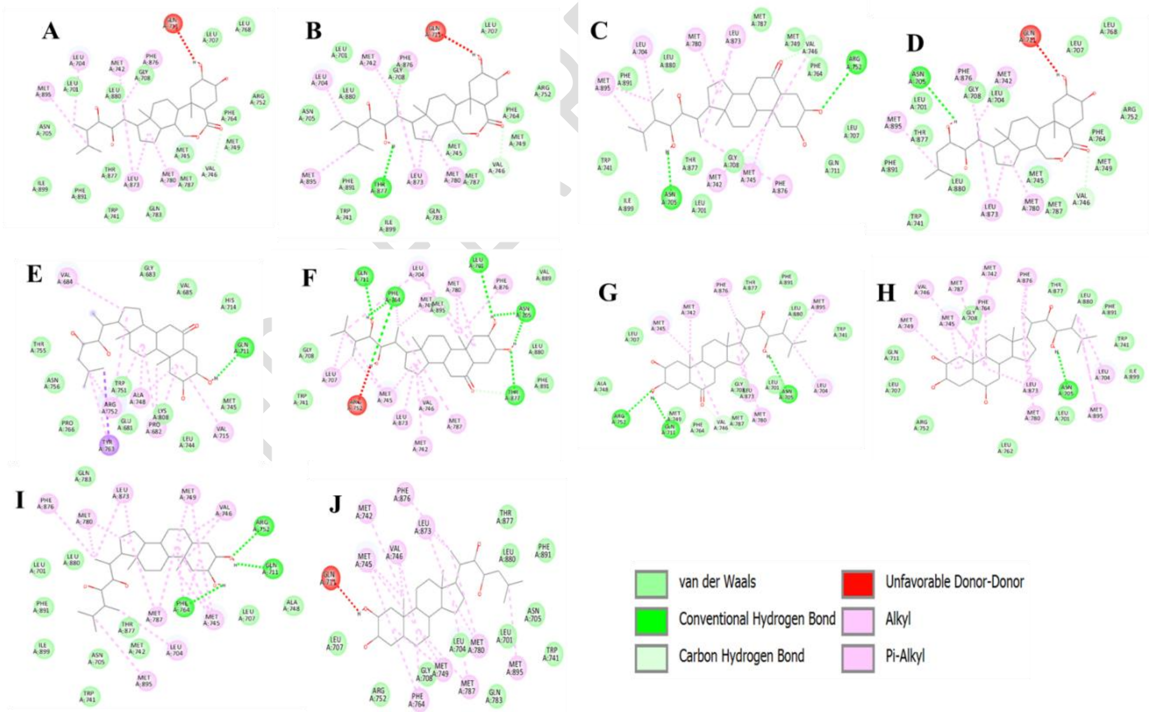


Figure 6. Shows A. Brassinolide, B. 28-Homobrassinolide, C. 28-Homocastasterone, D. 28-Norbrassinolide, E. 28-Norcastasterone, F. 24-Epicastasterone, G. 3,24-Diepicastasterone, H. 6αHydroxycastasterone, I. 6-Deoxocastasterone, J. 6-Deoxo-28-norcastasterone.

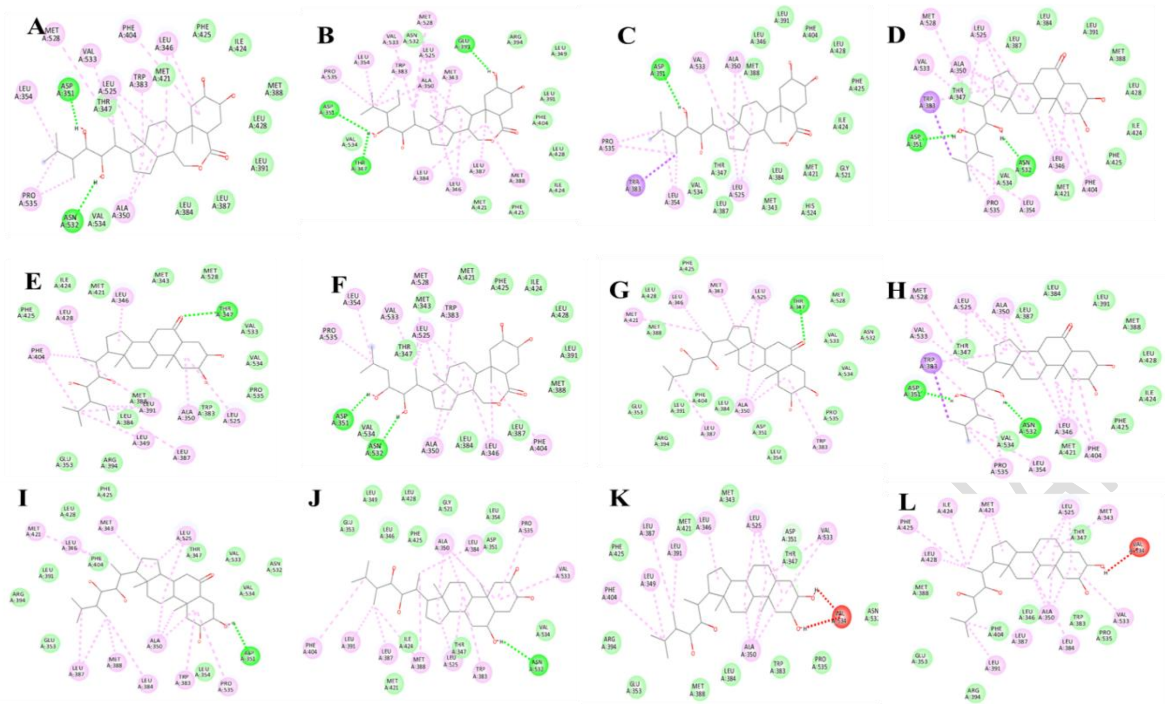


Figure 7. Shows A. Brassinolide, B. 28-Homobrassinolide, C. 24-Epibrassinolide, D. Castasterone, E. 28-Homocastasterone, F. 28-Norbrassinolide, G. 28-Norcastasterone, H. 24-Epicastasterone, I. 3,24-Diepicastasterone, J. 6αHydroxycastasterone, K. 6-Deoxocastasterone, L. 6-Deoxo-28-norcastasterone.

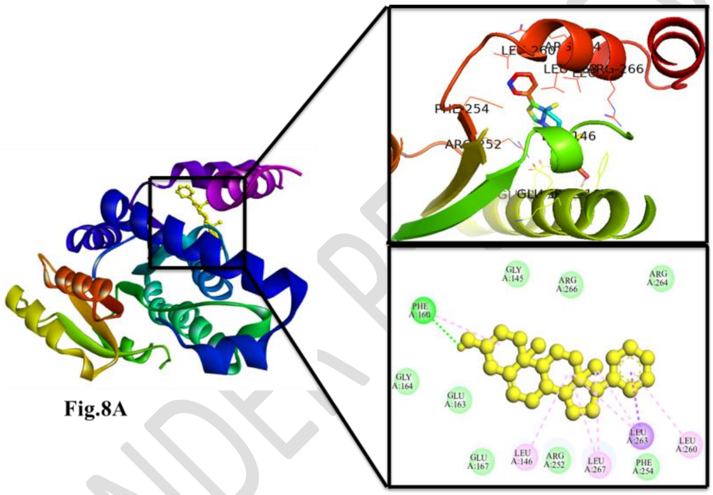


Fig.8A

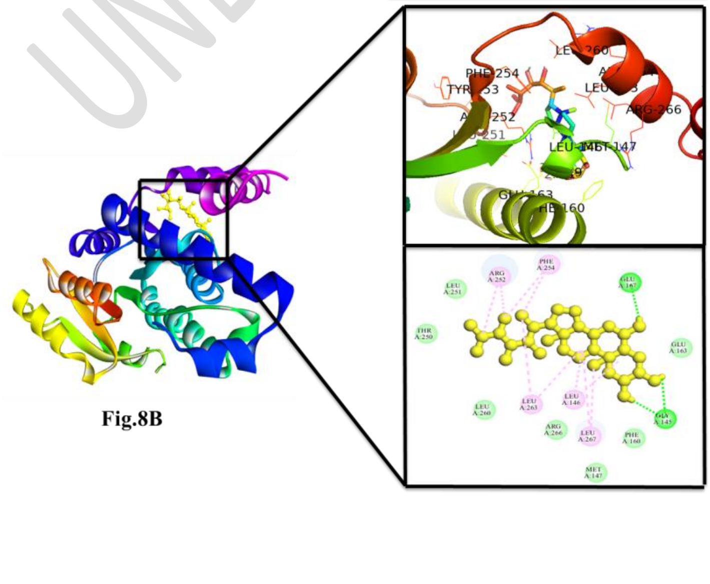


Fig.8B

Figure 8A. Showsthe3D and 2D presentation of abiraterone and 17βHSD enzyme amino acid interactions, **Figure.8B**.Shows the 3D and 2D presentation of 6α-Hydroxycastasterone and 17βHSD enzyme amino acid interactions.

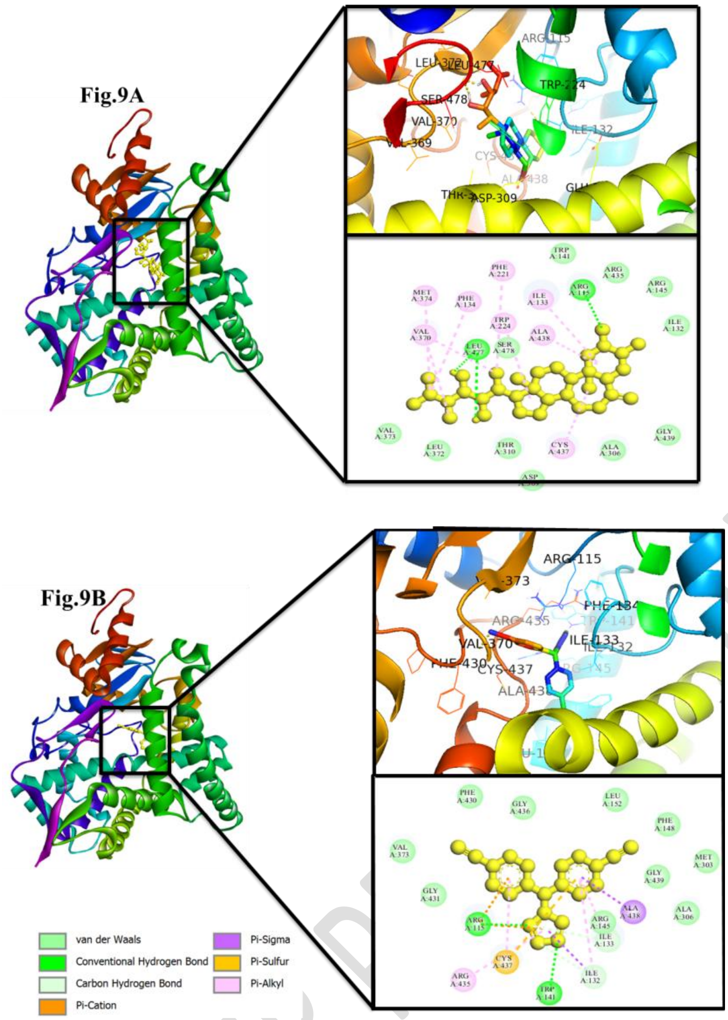
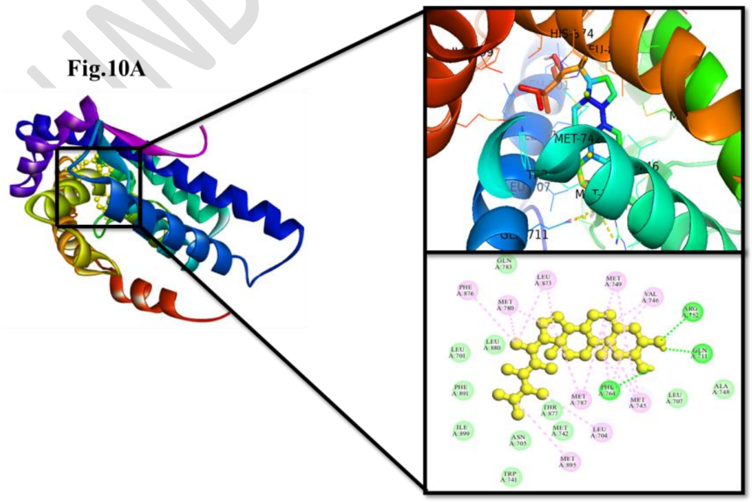


Figure 9A. Shows the 3D and 2D presentation of brassinolide and aromatase enzyme amino acid interactions, **Figure.9B.**Shows the 3D and 2D presentation ofletrozole and aromatase enzyme amino acid interactions.



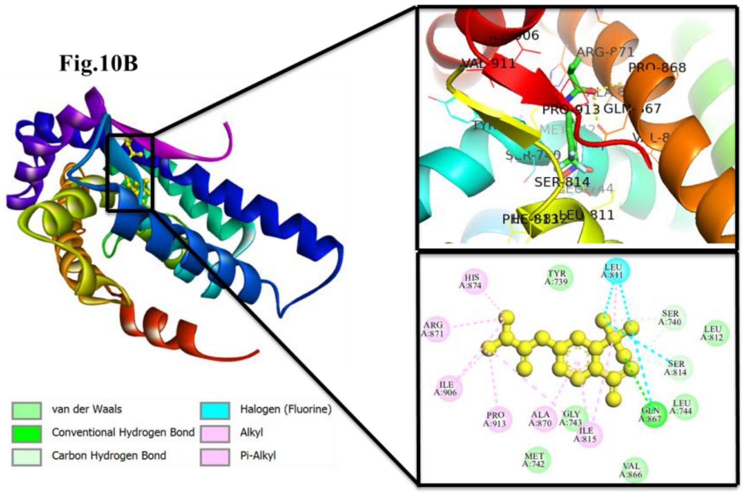
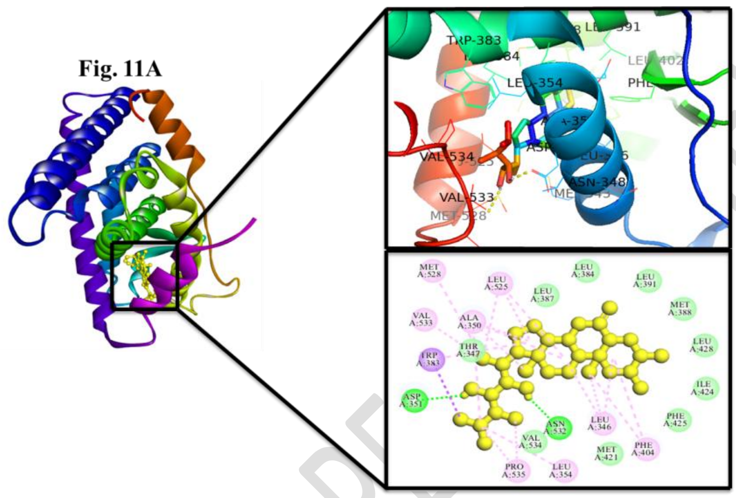


Figure 10A. Shows the 3D and 2D presentation of 6-Deoxocastasterone and androgen receptor amino acid interactions, **Figure.10B.**Shows the 3D and 2D presentation of Flutamide and androgen receptor amino acid interactions.



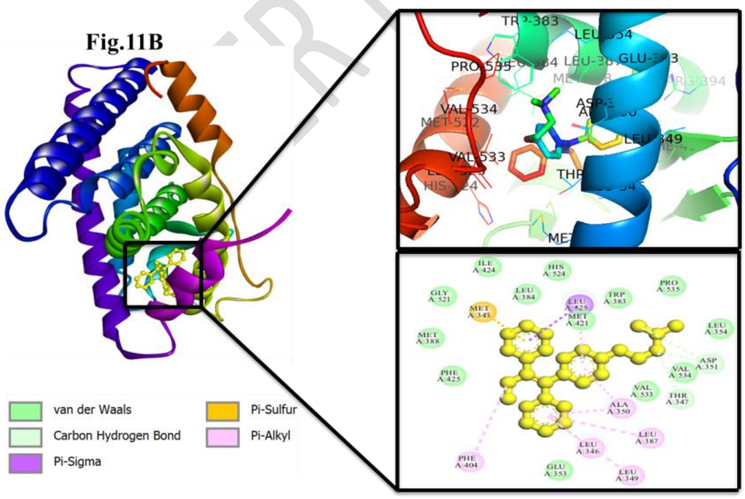


Figure 11A. Shows the 3D and 2D presentation of castasterone and androgen receptor amino acid interactions, **Figure.11B.**Shows the 3D and 2D presentation of tamoxifen and androgen receptor amino acid interactions.