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Putative ovarian steroidogenic effect of phyto oxysterol in Polycystic Ovary Syndrome an In Silico study

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Abstract:

Steroidogenesis in ovarian tissue is subject to obtainability of cholesterol besides the catalytic activity of HMGCoA reductase, 17β-hydroxysteroid dehydrogenase, aromatase and tissue levels of follicular stimulating hormone, luteinizing hormone. Polycystic ovary syndrome (PCOS) is a multifactorial resulted to increased level of testosterone and estradiol caused to transactivation of membrane bound androgen and estrogen receptors resulted to several clinical indices. Ovarian cholesterol, estrogen and testosterone levels are regulated by oxycholesterol through inhibition of key regulatory enzyme catalytic activity. Phyto oxysterol consumed by human through diet, however, role of phyto oxysterol in ovarian steroidogenesis and androgen, estrogen receptor was therefore investigated using In Silico molecular docking. Phyto oxysterol, enzyme and receptor three dimensional structure retrieved from chemical and protein data base. Further molecular docking analyses were performed using AutoDock 4.0 software. Phyto oxysterol exhibits high binding affinity on HMGCoA reductase, 17β-hydroxysteroid dehydrogenase, aromatase and androgen and estrogen receptor compared to conventional drugs. Current investigation suggested that daily intake of dietary oxysterol can be potentially modulating ovarian steroidogenesis and down regulating androgen and estrogen receptor transactivation resulted to ameliorating PCOS clinical complication.

Keywords: Oxysterol, Sterioidogenesis, PCOS, Molecular docking.

Introduction

Oxygenation forms of natural cholesterol called oxysterol (oxycholesterol) are generated in tissues through free-radical or cytochrome P450 enzymatic reactions. Oxysterols exerts biological effects in mammalians such as metabolic, immune and hormone regulations through activation of receptors by which modulating enzyme catalytic activity and gene expression in tissues resulted to bio-physiological changes in tissue (1). However, oxysterols derived from dietary plants with beneficial effects on health is a novel area of translational research.

In such an effort, we reported that enzymes and viral multiplications inhibitory effects of phyto oxysterols by using In Silico approach (2). Phyto oxysterol dolicholide, 2-Deoxy-25-Methyldolichosterone, Typhasterol, Teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone and 6-Deoxo-28-nortyphasterol are originate from plants and consumed by humans daily through a plant based diet. These molecules belong to the brassinosteroid hormone family in plant kingdom and act as bio-physiological factor which modulating the seed sprouting to healthy seed development. Nevertheless, phyto oxysterols have displayed structural resemblances with animals and insects oxysterols. Humans are exposed to animal and plant oxysterol day to day through diet (3). Thoughtful the effect of ubiquitously existing phyto oxysterol on ovarian sterioidogenesis and estrogen receptors signaling in polycystic ovarian syndrome (PCOS) is currently of interest.

PCOS is a multifactorial endocrine syndrome resulted to hyperandrogenism, polycystic ovary and anovulation leads to amenorrhoea, androgenic alopecia, clitoromegaly, hirsutism and infertility. Hence, indicative of hyperandrogenism with increased level of estrogen resulted to feedback regulated by elevating luteinizing hormone (LH) and reduction of follicular stimulating hormone (FSH) release from the anterior region of pituitary under GnRH stimulation (4). The reduced ovarian FSH obstructs the follicular maturation in ovary resulted to anovulation and along with prolonged increased estrogen act on endometrial cells resulted to endometrial hyperplasia (5).

Ovary sterioidogenesis are subjected to the obtainability of cholesterol in ovarian cells besides HMGCoA reductase, 17-beta hydroxysteroid dehydrogenase (17b-HSD), aromatase catalytic activity are major regulatory steps in ovarian steroidogenic pathway. Hence, targeting reducing the estrogen synthesis with inhibition of steroidogenic enzymatic catalyzing steps in synthesis of testosterone and estrogen along with inhibition of the androgen and estrogen receptors transactivation is another target for augmentation of FSH secretions and inhibiting obstacles in PCOS (6). In the current In Silico study, we investigate the anti-steroidogenic potential of plant oxysterol against HMGCoA reductase, 17βHSD, aromatase, androgen and estrogen receptor as target proteins in PCOS.

Methodology

Ligand preparation

Three-dimensional structures of Phyto oxysterol, namely, dolicholide (CID: 11038144), 2-Deoxy-25-Methyldolichosterone (CID: 14607938), typhasterol (CID: 13475120), teasterone (CID: 13475125), 28-Homoteasterone (CID: 154735171), 28-Nortyphasterol (CID: 13845862), 6-Deoxoteasterone (CID: 11144580), 6-Deoxo-28-nortyphasterol (CID: 102023289), simvastatin (CID: 54454), abiraterone (CID: 132971), letrozole (CID: 3902), flutamide (CID: 3397) and tamoxifen (CID: 2733526) were retrieved from PubChem database as .SDF format (http://pubchem.ncbi.nlm.nih.gov/). Followed by .SDF format were converted to .mol2 file format and molecules were optimized using AutoDock 4.0 program ligand preparation script. The ligands were prepared for molecular docking by means of torsion tree, detecting root were set than saved in .pdbqt format (Fig.1) (2).

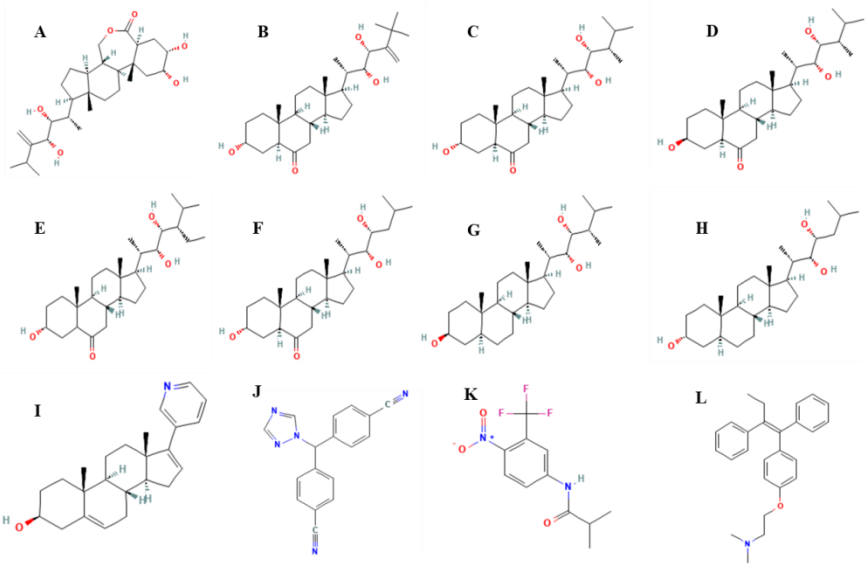


Figure 1. A. Dolicholide, B. 2-Deoxy-25-Methyldolichosterone, C. typhasterol, D. teasterone, E. 28-Homoteasterone, F. 28-Nortyphasterol, G. 6-Deoxoteasterone, H. 6-Deoxo-28-nortyphasterol, I. abiraterone, J. letrozole, K. flutamide, L. tamoxifen.

Protein preparation

3D structures of HMGCoA reductase (PDB ID: 1HW9), aromatase (PDB ID: 5JKV), 17βHSD (PDB ID: 1FDS), androgen receptor (PDB ID: 1E3G) and estrogen receptor (PDB ID: 3ERT) were retrieved from Protein Data Bank (http://www.rcsb.org). AutoDock version 4.0. software facilitated the protein structure preparation for molecular docking. Protein structure preparation comprising deletion of ligand, ions and water molecules followed by Kollman charges added than polar hydrogen atoms addition and saved the protein in .pdb file format for further docking (2,7).

Grid box generation

Three-dimensional structure of ligand and protein were together form a grid. Grid points X = 60, Y = 60 and Z = 60 axis fixed for docking and grid file format generated by “grid generation panel” in AutoDock version 4.0 (2,7).

Molecular docking simulation

Protein-ligand molecular docking was achieved by using AutoDock software version 4.0. For each ligand, 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. Numerous docked conformations were generated and 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 interactions between the target ligand and amino acid residues of the ligand binding cavity of protein were analyzed using BIOVIA Discovery Studio 2021 script (7).

Results

The enzyme HMGCoA reductase binding interactions with dietary phyto oxysterol were studied by In Silico docking simulations. Based on docking energies, teasterone, 6-Deoxoteasterone and 6-Deoxo-28-nortyphasterol are exhibited binding affinity -9.43, -9.69 and -9.34 Kcal/mole respectively as compared to simvastatin presented lower binding affinity -9.29 Kcal/mol with HMGCoA reductase (Table 1 and 6).

Teasterone forms hydrogen bond with ALA751, ALA753, ALA754, GLY773, ASN776 amino acid residues, while it forms Van der waals interaction with ALA694, ALA695, TRP698, GLY748, ASN750, HIS752, ASN755, VAL772, SER775 amino acid residues and PRO693, ILE746 amino acid residues form Alkyl/π-Alkyl interactions with HMGCoA reductase enzyme catalytic side (Table 6). While the oxysterol, 6-Deoxoteasterone, forms hydrogen bond with ASN658, ILE762, GLN766 amino acid residues, while it forms Van der waals interaction with CYS526, GLY656, MET657, MET659, TYR761, GLY765, ASP767, GLY807, GLY808, GLN814 amino acid residues and MET655, ILE762 amino acid residues form Alkyl/π-Alkyl interactions (Table 1). 6-Deoxo-28-nortyphasterol forms hydrogen bond with GLN766 amino acid residue, while it forms Van der waals interaction with GLY656, MET657, ASN658, MET659, TYR761, ILE762, GLY765, ASP767, GLY803, VAL805, GLY806, GLY807, GLY808, THR809, LEU811, GLN814 amino acid residues and ALA654, MET655 amino acid residues form Alkyl/π-Alkyl interactions with HMGCoA reductase enzyme catalytic side (Table 1). Likewise, the binding studies performed between simvastatin and HMGCoA reductase, indicates simvastatin interacting with amino acid residues CYS526, MET655, GLY808 via hydrogen bond and with amino acid residues GLY656, MET657, ASN658, CYS691, GLY765, GLN766, ASP767, GLY803, THR804, GLY807, THR809 via Van der waals interaction and ALA654 residue via Alkyl/π-Alkyl interactions with HMGCoA reductase (Table 6).

Table 1. Oxysterol and HMGCoA reductase interactions.

HMGCoA reductase (1HW9)	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl
Dolicholide	-7.12	ILE746, GLY747, GLY748, ASN750, ALA753, ASN755, ASN771, SER775, CYS777.	THR758, ASN776.	PRO693, ALA754, VAL772.
2-Deoxy-25-Methyldolichosterone	-9.12	CYS526, ILE536, ALA654, MET657, ASN658, TYR761, GLY765, ASP767, ALA768, GLY806, GLY807, GLY808.	GLY656, GLN766.	MET655, ILE762.
Typhasterol	-6.47	GLY748, ASN750, ALA768, SER775, ASN776, CYS777.	GLY747, THR758, ASN771.	ALA754, ALA769, VAL772.
Teasterone	-9.43	ALA694, ALA695, TRP698, GLY748, ASN750, HIS752, ASN755, VAL772, SER775.	ALA751, ALA753, ALA754, GLY773, ASN776.	PRO693, ILE746.
28-Homoteasterone	-7.56	GLY559, ALA694, ILE746, GLY747, ASN750, ASN771, VAL772, SER775, CYS777.	GLY748, ASN755, ASN776.	PRO693, ASN754.
28-Nortyphasterol	-8.56	ALA694, SER745, GLY747, GLY748, ASN750, HIS752, ALA753, GLY773, SER775, LEU853.	ILE746, ALA751, ASN755, ASN776.	PRO693, ASN754, VAL772.
6-Deoxoteasterone	-9.69	CYS526, GLY656, MET657, MET659, TYR761, GLY765, ASP767, GLY807, GLY808, GLN814.	ASN658, ILE762, GLN766.	MET655, ILE762.
6-Deoxo-28-nortyphasterol	-9.34	GLY656, MET657, ASN658, MET659, TYR761, ILE762, GLY765, ASP767, GLY803, VAL805, GLY806, GLY807, GLY808, THR809, LEU811, GLN814.	GLN766.	ALA654, MET655.

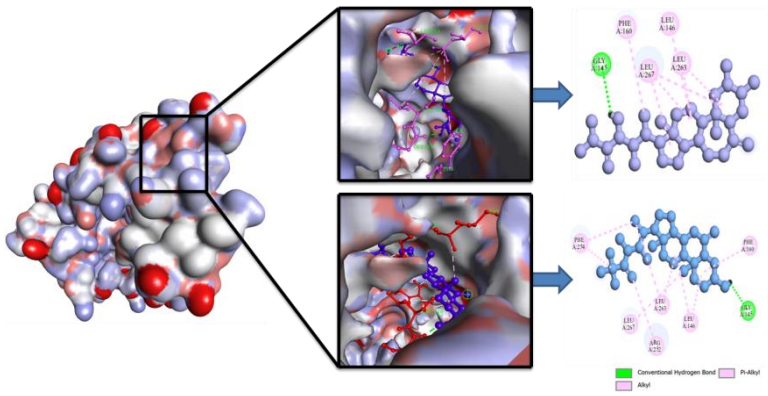


Figure 2. 2D and 3D presentation of 17βHSD enzyme and dolicholide, 2-Deoxy-25 Methyldolichosterone interactions.

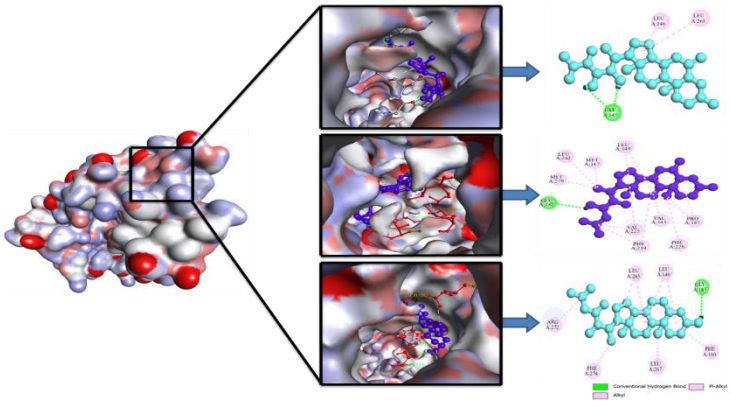


Figure 3. 2D and 3D presentation of ligands typhasterol, 28-Nortyphasterol and 6-Deoxo-28-nortyphasterol and 17 βHSD interactions.

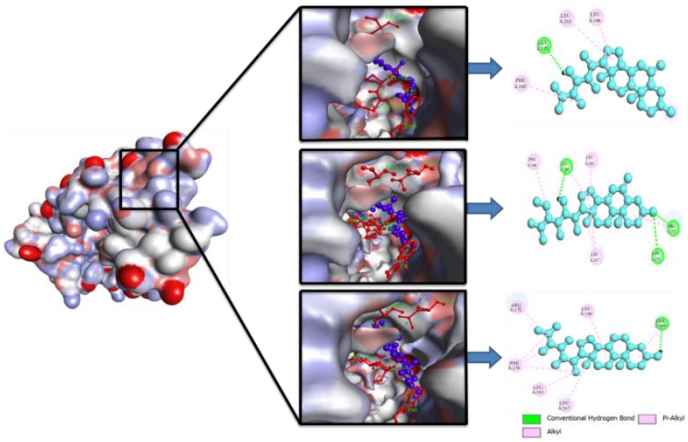


Figure 4. 2D and 3D presentation of ligands teasterone, 28-Homoteasterone, 6-Deoxoteasterone and 17 βHSD interactions.

The enzyme 17βHSD binding interactions with dietary phyto oxysterol were studied by In Silico docking simulations. Based on docking energies, dolicholide, 2-Deoxy-25-Methyldolichosterone, typhasterol, teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone, 6-Deoxo-28-nortyphasterol are exhibited binding affinity -7.37, -7.71, -8.26, -7.72, -7.98, -10.61, -7.40 and -7.91 Kcal/mole respectively as compared to abiraterone presented binding affinity -7.68, Kcal/mol with 17βHSD (Table 2 and 6).

The dolicholide forms hydrogen bond with GLY145 amino acid residue, while it forms Van der waals interaction with GLU163, GLY164, GLU167, ARG252, PHE254, LEU260, ARG264, ARG266 amino acid residues and amino acid residues LEU146, PHE160, LEU263, LEU267 form Alkyl/π-Alkyl interactions with 17βHSD enzyme. While the oxysterol, 2-Deoxy-25-Methyldolichosterone forms hydrogen bond with GLY145 amino acid residue, while it forms Van der waals interaction with GLU163, GLU167, THR250, LEU251, TYR253, ARG266 amino acid residues and amino acid residues LEU146, PHE160, ARG252, PHE254, LEU263, LEU267 form Alkyl/π-Alkyl interactions with 17βHSD enzyme. Similarly, typhasterol forms hydrogen bond with GLY145 amino acid residue, while it forms Van der waals interaction with PHE160, GLU163, GLY164, GLU167, PHE254, ARG266, LEU267 amino acid residues and amino acid residues LEU146, LEU263 form Alkyl/π-Alkyl and GLN711 and ARG752 form unfavorable interactions. The teasterone forms hydrogen bond with GLY145 amino acid residue, while it forms Van der waals interaction with GLU163, GLY164, GLU167, PHE254, LEU260, LEU267 amino acid residues and amino acid residues LEU146, PHE160, LEU263 form Alkyl/π-Alkyl interactions in catalytic side of the enzyme. 28-Homoteasterone forms hydrogen bond with LEU146 amino acid residue, while it forms Van der waals interaction with GLY145, MET147, GLU163, GLY164, GLY167, THR250, PHE254, ARG266 amino acid residues and amino acid residues PHE160, LEU263, LEU267 form Alkyl/π-Alkyl interactions. The 28-Nortyphasterol forms hydrogen bond with GLU282 amino acid residue, while it forms Van der waals interaction with SER142, GLY144, ASN152, TYR155, CYS185, GLY186, TYR218, HIS221, GLU228, ALA229, ARG258, VAL282 amino acid residues and amino acid residues VAL143, MET147, LEU149, PRO187, VAL225, PHE226, PHE259, LEU262, MET272 form Alkyl/π-Alkyl interactions (Table 2 and Fig

3). The 6-Deoxoteasterone forms hydrogen bond with PHE160 amino acid residue, while it forms Van der waals interaction with GLY145, ALA161, GLU163, GLY164, GLU167, THR250, LEU251, ARG266 amino acid residues and amino acid residues LEU146, PHE160, LEU263, LEU267 form Alkyl/π-Alkyl interactions (Table 2 and Fig 2-4). Likewise, the binding studies performed between abiraterone and 17βHSD enzyme, indicates abiraterone interacting with amino acid residues PHE160 via 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 interaction with 17βHSD enzyme (Table 6 and Fig 14).

Table 2. Oxysterol and 17 Beta-Hydroxysteroid-dehydrogenase interactions.

17βhydroxysteroid dehydrogenase (1FDS)	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl
Dolicholide	-7.37	GLU163, GLY164, GLU167, ARG252, PHE254, LEU260, ARG264, ARG266.	GLY145	LEU146, PHE160, LEU263, LEU267.
2-Deoxy-25-Methyldolichosterone	-7.71	GLU163, GLU167, THR250, LEU251, TYR253, ARG266.	GLY145.	LEU146, PHE160, ARG252, PHE254, LEU263, LEU267.
Typhasterol	-8.26	PHE160, GLU163, GLY164, GLU167, PHE254, ARG266, LEU267.	GLY145.	LEU146, LEU263.
Teasterone	-7.72	GLU163, GLY164, GLU167, PHE254, LEU260, LEU267.	GLY145.	LEU146, PHE160, LEU263.
28-Homoteasterone	-7.98	GLY145, MET147, GLU163, GLY164, GLY167, THR250, PHE254, ARG266.	LEU146.	PHE160, LEU263, LEU267.
28-Nortyphasterol	-10.61	SER142, GLY144, ASN152, TYR155, CYS185, GLY186, TYR218, HIS221, GLU228, ALA229, ARG258, VAL282.	GLU282.	VAL143, MET147, LEU149, PRO187, VAL225, PHE226, PHE259, LEU262, MET272.
6-Deoxoteasterone	-7.40	GLY145, ALA161, GLU163, GLY164, GLU167, THR250, LEU251, ARG266.	PHE160	LEU146, PHE160, LEU263, LEU267
6-Deoxo-28-nortyphasterol	-7.91	GLU163, GLU167, THR250, LEU251, ARG266.	GLY145.	LEU146, PHE160, ARG252, PHE254, LEU263.

The aromatase binding interactions with phyto oxysterol were studied by In Silico docking simulations. Based on docking energies obtained, aromatase exhibited binding affinity towards dolicholide, 2-Deoxy-25-Methyldolichosterone, typhasterol, teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone and 6-Deoxo-28-nortyphasterol is -12.01, -11.28, -10.97, -11.11, -10.90, -11.43, -11.34 and -11.10 Kcal/mole respectively as compared to letrozole presented binding affinity -7.93 Kcal/mol with aromatase (Table 3).

In aromatase enzyme interaction, ligand dolicholide interacted with ARG115, CYS43, GLY439, LEU477 residues via hydrogen bond and ILE132, ILE133, TRP141, ARG145, ALA306, ASP309, THR310, VAL370, LEU372, VAL373, MET374, ARG435, ALA438, SER478 residues via Van der waals interaction and PHE134, PHE221, TRP224 residues via Alkyl/π-Alkyl interactions. 2-Deoxy-25-Methyldolichosterone interacted with LEU477 residue via hydrogen bond and ARG115, ILE132, PHE134, ARG145, ASP309, THR310, VAL373, MET374, SER473 residues via Van der waals interaction and residues ILE133, PHE221, TRP224, ALA306, VAL370, LEU372, CYS437 via Alkyl/π-Alkyl interactions. Typhasterol interacted with residues THR310, LEU372, CYS437 via hydrogen bond and amino acid residues ARG115, PHE203, TRP224, ASP309, VAL370, MET374, GLY439, ALA443 via Van der waals interaction and ILE133, PHE134, LEU152, ALA306, ALA307, MET311, VAL373, MET446 residues via Alkyl/π-Alkyl interactions. Teasterone interacted with residues ARG145 and LEU477 via hydrogen bond and amino acid residues ARG115, ILE132, ASP309, THR310, MET374, GLY436, GLY439, LEU477, SER478 via Van der waals interaction and PHE134, ILE133, PHE221, TRP224, ALA306, VAL370, LEU372, CYS437, ALA438 residues via Alkyl/π-Alkyl interactions. 28-Homoteasterone interacted with ASP371, ILE372, SER478, LEU477 residues via hydrogen bond and amino acid residues ARG115, ILE134, PHE134, ARG145, PHE221,

GLU302, MET303, THR310, ARG369, VAL373, CYS437 via Van der waals interaction and ILE133, ILU152, PHE148, TRP224, ALA306, VAL370, ALA438 residues via Alkyl/π-Alkyl interactions and amino acid residue GLY439 form unfavorable donor-donor band with aromatase enzyme. 28-Nortyphasterol interacted with LEU374, LEU477 residues via hydrogen bond and amino acid residues ARG115, ILE132, ARG145, ALA306, ASP309, VAL373, SER478 via Van der waals interaction and residues ILE133, PHE134, PHE221, TRP224, VAL370, MET374, CYS437, ALA438 via Alkyl/π-Alkyl interactions. 6-Deoxoteasterone interacted with residue LEU372 via hydrogen bond and amino acid residues ARG115, ILE134, PHE148, ILE152, PHE221, THR310, CYS437, SER478 via Van der waals interaction and residues ILE133, PHE134, TRP224, ALA306, VAL370, VAL373, MET374, ALA438, LEU477 via Alkyl/π-Alkyl interactions. 6-Deoxo-28-nortyphasterol interacted with residue THR310, GLY436 via hydrogen bond and amino acid residues ARG115, ILE132, ARG145, SER314, PRO429, ARG435 via Van der waals interaction and ILE133, MET311, MET364, PRO368, VAL369, VAL370, PHE430, CYS437, ALA438 residues via Alkyl/π-Alkyl interactions with phyto oxysterol (Table 3 and Fig 5-7). Likewise, the binding studies performed between leterozole and aromatase enzyme, indicates letrozole interacts with aromatase amino acid residues ARGY115, TRP141 via hydrogen bond, while ILE132, ILE133, ARG145, LEU152, MET303, ALA306, VAL373, PHE430, GLY431, GLY439 amino acid residues via Van der waals interactions and amino acid residues ARG435 via Alkyl/π-Alkyl interactions and amino acid residues CYS437, ALA438 via π-sigma interactions with phyto oxysterol (Table 6 and Fig 15).

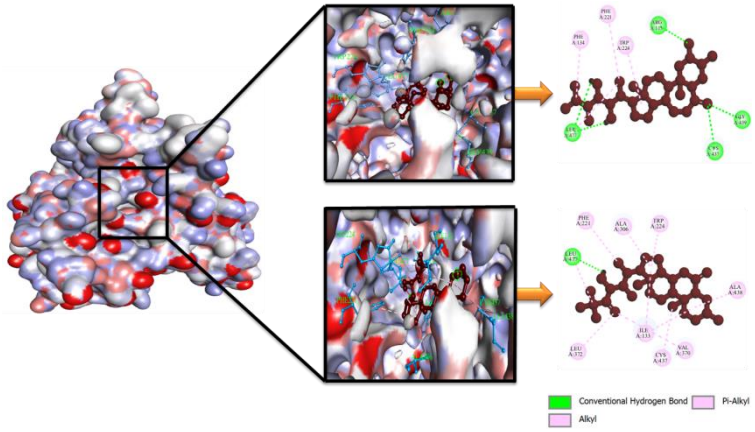


Figure 5. 2D and 3D presentation of aromatase and dolicholide, 2-Deoxy-25-Methyldolichosterone ligands interactions

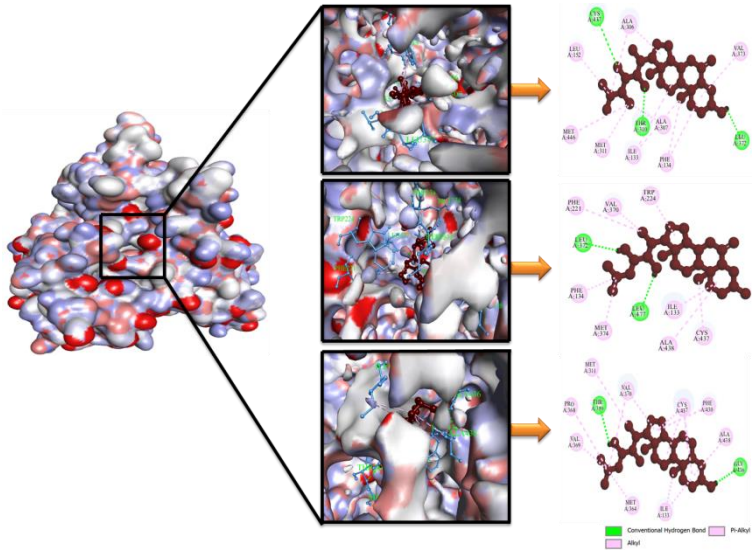


Figure 6. 2D and 3D presentation of typhasterol, 28-Nortyphasterol and 6-Deoxo-28-nortyphasterol and aromatase interactions.

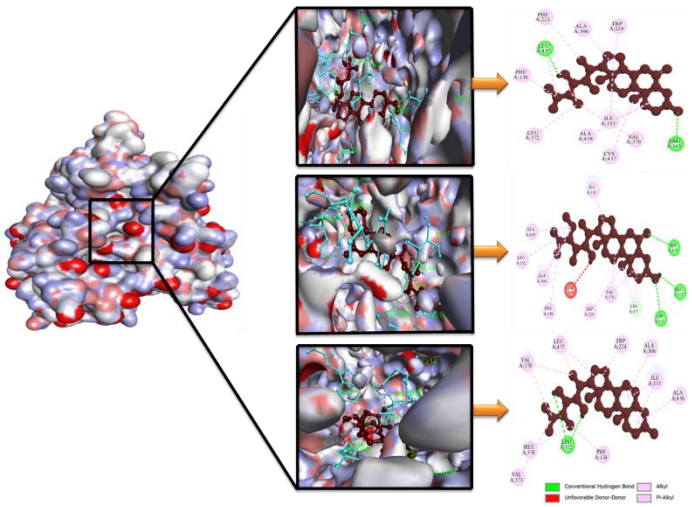


Figure 7. 2D and 3D presentation of ligands teasterone, 28-Homoteasterone, 6-Deoxoteasterone and aromatase interactions.

In the current In Silico study, androgen receptor was docked with the phyto oxysterol and the docking scores of dolicholide, 2-Deoxy-25-Methyldolichosterone, typhasterol, teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone, 6-Deoxo-28-nortyphasterol against androgen receptor is -5.54, -6.49, -8.25, -8.70, -0.8, -7.84, -7.41 and -9.16 Kcal/mole respectively as compared to flutamide presented binding affinity -1.83 Kcal/mol with androgen receptor (Table 4 and 6).

Table 4. Oxysterol and androgen receptor interaction.

Androgen receptor (1E3G)	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl
Dolicholide	-5.54	GLY708, TRP741, LEU768, MET787, PHE876, LEU880, MET895.	LEU701, ASN705, GLN711, PHE764, THR877.	LEU704, LEU707, MET742, MET745, VAL746, MET749, MET780, LEU873.
2-Deoxy-25-Methyldolichosterone	-6.49	ASN705, GLY708, GLN711, MET742, VAL746, TYR763, ALA765, MET787, PHE876, THR877.	LEU701	LEU704, LEU707, MET745, MET749, PHE764, MET780, LEU873, LEU880, PHE891, MET895.
Typhasterol	-8.25	ASN705, GLY708, LEU768, PHE891, MET895.	THR877	LEU701, LEU704, LEU707, MET742, MET745, VAL746, MET749, PHE764, MET780, MET787, LEU873, PHE876, LEU880.
Teasterone	-8.70	LEU701, ASN705, LEU707, GLY708, GLN711, MET742, VAL746, ALA748, GLN783, MET787, GLU872, THR877, MET895.	MET745, ARG752, MET780.	LEU704, MET749, PHE764, GLU872, THR877, MET895.
28-Homoteasterone	-8.80	LEU701, ASN705, GLY708, GLN711, VAL746, ALA748, LEU768, GLN783, PHE876, THR877, LEU880, ILE899, MET895.	LEU873	LEU704, LEU707, TRP741, MET742, MET745, MET749, MET749, MET780, MET787, PHE891.
28-Nortyphasterol	-7.84	LEU701, LEU704, ASN705, GLY708, GLN711, TRP741, MET742, ARG752, ALA765, GLN783, LEU880, PHE891.	VAL746, PHE764, THR877.	LEU707, MET745, MET749, MET780, MET787, LEU873, PHE876, MET895.
6-Deoxoteasterone	-7.41	LEU701, ASN705, GLY708, MET742, VAL746, ALA748, GLN783, MET787, GLU872, MET895.	GLN711, ARG752, MET780.	LEU704, LEU707, MET745, MET749, PHE764, LEU880, PHE891.
6-Deoxo-28-nortyphasterol	-9.16	ASN705, GLY708, TRP741, PHE876, PHE891.	GLN711, PHE764, THR877.	LEU701, LEU704, LEU707, MET742, MET745, VAL746, MET749, MET780, MET787, LEU873, LEU880.

In androgen receptor interaction, ligand dolicholide interacted with residues LEU701, ASN705, GLN711, PHE764, THR877 via hydrogen bond and GLY708, TRP741, LEU768, MET787, PHE876, LEU880, MET895 residues via Van der waals interaction and LEU704, LEU707, MET742, MET745, VAL746, MET749, MET780, LEU873 residues via Alkyl/π-Alkyl interactions and amino acid residue ASN705, ARG752 form unfavorable donor and acceptor interaction with receptor. 2-Deoxy-25-Methyldolichosterone interacted with residue LEU701 via hydrogen bond and residues ASN705, GLY708, GLN711, MET742, VAL746, TYR763, ALA765, MET787, PHE876, THR877 via Van der waals interaction and residues LEU704, LEU707, MET745, MET749, PHE764, MET780, LEU873, LEU880, PHE891, MET895 via Alkyl/π-Alkyl interactions and amino acid residue ARG752 via unfavorable donor-donor interactions. Typhasterol interacted with residue THR877 via hydrogen bond and residues ASN705, GLY708, LEU768, PHE891, MET895 via Van der waals interaction and residues LEU701, LEU704, LEU707, MET742, MET745, VAL746, MET749, PHE764, MET780, MET787, LEU873, PHE876, LEU880 via Alkyl/π-Alkyl interactions and amino acid residues GLN711 and ARG752 via unfavorable interactions (Table and Fig). Teasterone interacted with residues MET745, ARG752, MET780 via hydrogen bond and residues LEU701, ASN705, LEU707, GLY708, GLN711, MET742, VAL746, ALA748, GLN783, MET787, GLU872, THR877, MET895 via Van der waals interaction and residues LEU704, MET749, PHE764, GLU872, THR877, MET895 via Alkyl/π-Alkyl interactions and amino acid residue LEU873 via unfavorable interaction . 28-Homoteasterone interacted with residue LEU873 via hydrogen bond and residues LEU701, ASN705, GLY708, GLN711, VAL746, ALA748, LEU768, GLN783, PHE876, THR877, LEU880, ILE899, MET895 via Van der waals interaction and residues LEU704, LEU707, TRP741, MET742, MET745, MET749, MET749, MET780, MET787, PHE891 via Alkyl/π-Alkyl interactions. 28-Nortyphasterol interacted with residues VAL746, PHE764, THR877 via hydrogen bond and residues LEU701, LEU704, ASN705, GLY708, GLN711, TRP741, MET742, ARG752, ALA765, GLN783, LEU880, PHE891 via Van der waals interaction and residues LEU707, MET745, MET749, MET780, MET787, LEU873, PHE876, MET895 via Alkyl/π-Alkyl interactions. 6-Deoxoteasterone interacted with residues GLN711, ARG752, MET780 via hydrogen bond and residues LEU701, ASN705, GLY708, MET742, VAL746, ALA748, GLN783, MET787, GLU872, MET895 via Van der waals interaction and residues LEU704, LEU707,MET745, MET749, PHE764, LEU880, PHE891 via Alkyl/π-Alkyl interactions and amino acid residue PHE876, via π-sigma interaction and amino acid LEU873 form unfavorable interaction. 6-Deoxo-28-nortyphasterol interacted with residues GLN711, PHE764, THR877 via hydrogen bond and residues ASN705, GLY708, TRP741, PHE876, PHE891 via Van der waals interaction and residues LEU701, LEU704, LEU707, MET742, MET745, VAL746, MET749, MET780, MET787, LEU873, LEU880 via Alkyl/π-Alkyl interactions and amino acid residue ARG752 via unfavorable interaction (Table 4 and Fig 8, 9, 10). Likewise, the binding studies performed between flutamide and androgen receptor, indicates flutamide interacting with amino acid residues SER740, SER814, GLN867 via hydrogen bond and with amino acid residues THR739, MET742, GLY743, LEU744, LEU812, VAL866 via Van der waals interaction and LEU811, ILE815, ALA870,ARG871 HIS874, ILE906, PRO913 residues via Alkyl/π-Alkyl interactions with the phyto oxysterol (Table 6 and Fig 16).

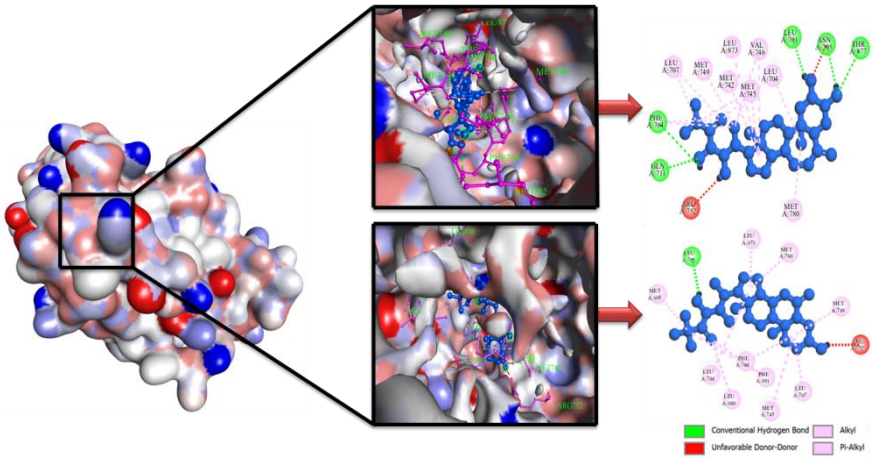


Figure 8. 2D and 3D presentation of androgen receptor and dolicholide, 2-Deoxy-25-Methyldolichosterone interactions.

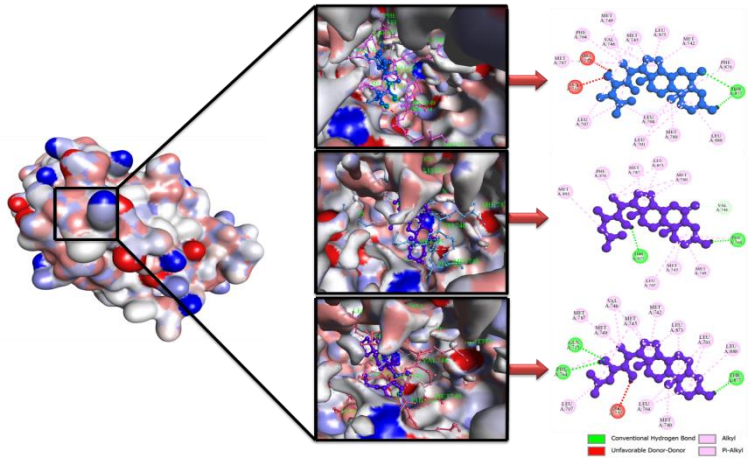


Figure 9. 2D and 3D presentation of ligands typhasterol, 28-Nortyphasterol and 6-Deoxo-28-nortyphasterol and androgen receptor interactions.

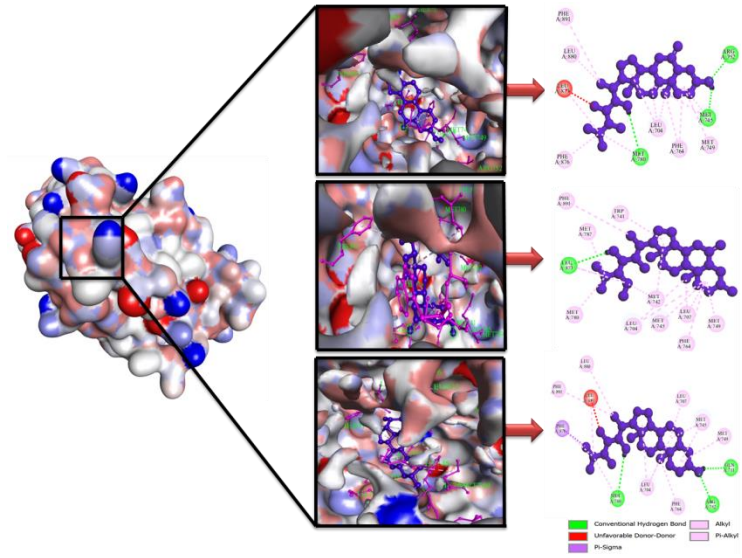


Figure 10. 2D and 3D presentation of teasterone, 28-Homoteasterone, 6-Deoxoteasterone and androgen receptor interactions.

In the current In Silico study, estrogen receptor was docked with the phyto oxysterol. The docking scores of dolicholide, 2-Deoxy-25-Methyldolichosterone, typhasterol, teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone, 6-Deoxo-28-nortyphasterol against estrogen receptor is -12.24, -12.25, -10.81, -12.24, -12.35, -12.18, -12.99 and -10.94 Kcal/mole respectively as compared to tamoxifen presented binding affinity -11.03 Kcal/mol with estrogen receptor (Table 5 and 6).

Table 5. Oxysterol and estrogen receptor interaction.

Estrogen receptor (3ERT)	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl
Dolicholide	-12.24	THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, PHE425, LEU428, VAL534.	ASP351, ASN532.	LEU346, ALA350, LEU354, TRP383, PHE404, LEU525, MET528, VAL533, PRO535.
2-Deoxy-25-Methyldolichosterone	-12.25	MET343, THR347, LEU354, LEU384, LEU387, MET388, LEU391, ILE424, PHE425, VAL534.	ASP351, ASN532.	LEU346, ALA350, PHE404, MET421, LEU525, MET528, VAL533, PRO535.
Typhasterol	-10.81	THR347, ARG394, PHE404, MET421, PHE425, LEU428, VAL534.	ASP351, LEU387.	MET343, LEU346, ALA350, LEU354, TRP383, MET388, LEU391, LEU525, MET528, VAL533, PRO535.
Teasterone	-12.24	THR347, GLU353, LEU354, ARG394, PHE425, LEU428, ASN532, VAL533, VAL534.	ASP551	MET343, LEU346, ALA350, TRP383, LEU384, LEU387, MET388, LEU391, PHE404, MET421, LEU525, PRO535.

28-Homoteasterone	-12.35	THR347, GLU353, ARG394, PHE404, MET421, ILE424, PHE425, LEU428, ASN532, VAL534.	ASP351	MET343, LEU346, ALA350, LEU354, TRP383, LEU384, LEU387, MET388, LEU391, LEU525, VAL533, PRO535, LEU554.
28-Nortyphasterol	-12.18	MET343, THR347, LEU354, LEU384, LEU387, MET388, LEU391, ILE424, PHE425, LEU428, ASN532.	MET421	LEU346, ALA350, PHE404, LEU525, MET528, VAL533, PRO535.
6-Deoxoteasterone	-12.99	MET343, LEU346, THR347, LEU349, ASP351, GLU353, ARG394, MET421, ILE424, PHE425, LEU428, VAL534.	ASN532	ALA350, TRP383, LEU384, LEU387, MET388, LEU391, PHE404, LEU525, VAL533, PRO535.
6-Deoxo-28-nortyphasterol	-10.94	MET343, THR347, LEU387, LEU391, LEU402, ILE424, LEU428, ASN532.	ASP351, MET421.	LEU346, ALA350, LEU354, LEU384, MET388, PHE404, PHE425, LEU525, MET528, VAL533, PRO535.

Dolicholide interacted with residues ASP351 and ASN532 via hydrogen bond and residues THR347, LEU384, LEU387, MET388, LEU391, MET421, ILE424, PHE425, LEU428, VAL534 via Van der waals interaction and residues LEU346, ALA350, LEU354, TRP383, PHE404, LEU525, MET528, VAL533, PRO535 via Alkyl/π-Alkyl interactions. 2-Deoxy-25-Methyldolichosterone interacted with residues ASP351, ASN532 via hydrogen bond and residues MET343, THR347, LEU354, LEU384, LEU387, MET388, LEU391, ILE424, PHE425, VAL534 via Van der waals interaction and residues LEU346, ALA350, PHE404, MET421, LEU525, MET528, VAL533, PRO535 via Alkyl/π-Alkyl interactions and amino acid residue TRP383 form π-sigma interaction. Typhasterol interacted with residues ASP351, LEU387 via hydrogen bond and residues THR347, ARG394, PHE404, MET421, PHE425, LEU428, VAL534 via Van der waals interaction and residues MET343, LEU346, ALA350, LEU354, TRP383, MET388, LEU391, LEU525, MET528, VAL533, PRO535 via Alkyl/π-Alkyl interactions. Teasterone interacted with residue ASP551 via hydrogen bond and residues THR347, GLU353, LEU354, ARG394, PHE425, LEU428, ASN532, VAL533, VAL534 via Van der waals interaction and residues MET343, LEU346, ALA350, TRP383, LEU384, LEU387, MET388, LEU391, PHE404, MET421, LEU525, PRO535 via Alkyl/π-Alkyl interactions. 28-Homoteasterone interacted with residue ASP351 via hydrogen bond and residues THR347, GLU353, ARG394, PHE404, MET421, ILE424, PHE425, LEU428, ASN532, VAL534 via Van der waals interaction and residues MET343, LEU346, ALA350, LEU354, TRP383, LEU384, LEU387, MET388, LEU391, LEU525, VAL533, PRO535, LEU554 via Alkyl/π-Alkyl interactions. 28-Nortyphasterol interacted with residue MET421 via hydrogen bond and residues MET343, THR347, LEU354, LEU384, LEU387, MET388, LEU391, ILE424, PHE425, LEU428, ASN532 via Van der waals interaction and residues LEU346, ALA350, PHE404, LEU525, MET528, VAL533, PRO535 via Alkyl/π-Alkyl interactions and amino acid residue TRP383, VAL534 form unfavorable interaction. 6-Deoxoteasterone interacted with residue ASN532 via hydrogen bond and residues MET343, LEU346, THR347, LEU349, ASP351, GLU353, ARG394, MET421, ILE424, PHE425, LEU428, VAL534 via Van der waals interaction and residues ALA350, TRP383, LEU384, LEU387, MET388, LEU391, PHE404, LEU525, VAL533, PRO535 via Alkyl/π-Alkyl interactions. 6-Deoxo-28-nortyphasterol interacted with residues ASP351, MET421 via hydrogen bond and residues MET343, THR347, LEU387, LEU391, LEU402, ILE424, LEU428, ASN532 via Van der waals interaction and residues LEU346, ALA350, LEU354, LEU384, MET388, PHE404, PHE425, LEU525, MET528, VAL533, PRO535 via Alkyl/π-Alkyl interactions and amino acid residue TRP383 form π-sigma and VAL534 amino acid form unfavorable interaction (Table 5 and Fig 11-13).

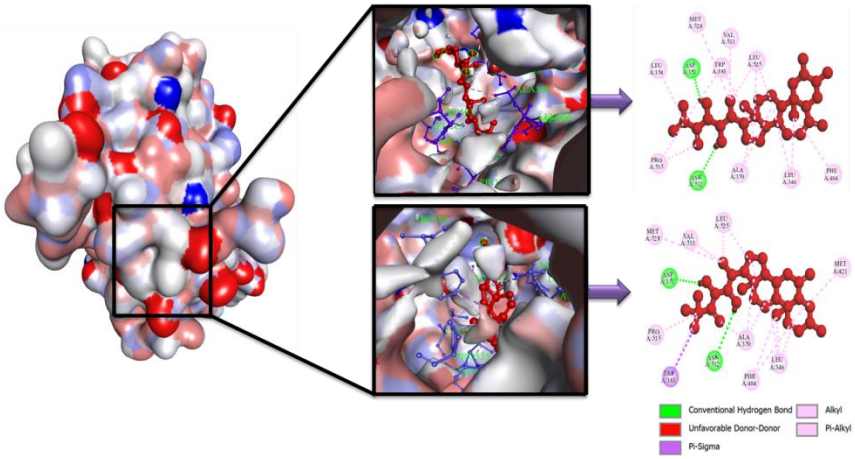


Figure 11. 2D and 3D presentation of estrogen receptor and dolicholide, 2-Deoxy-25-Methyldolichosterone interactions.

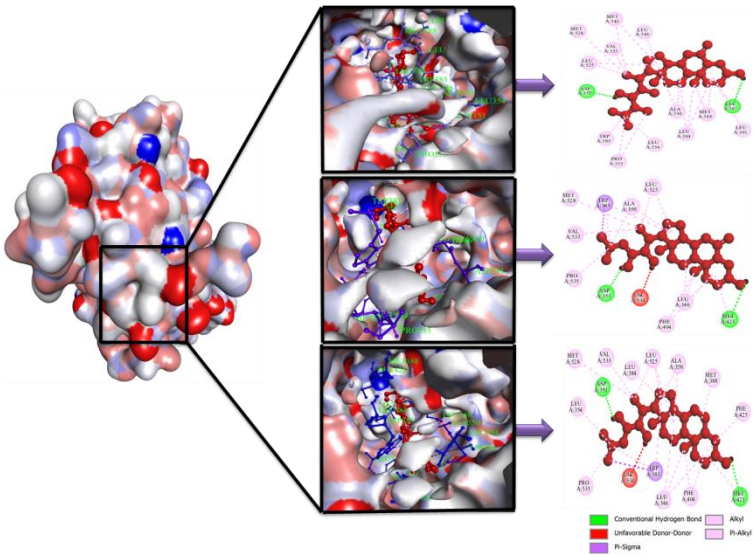


Figure 12. 2D and 3D presentation of typhasterol, 28-Nortyphasterol and 6-Deoxo-28-nortyphasterol and estrogen receptor interactions.

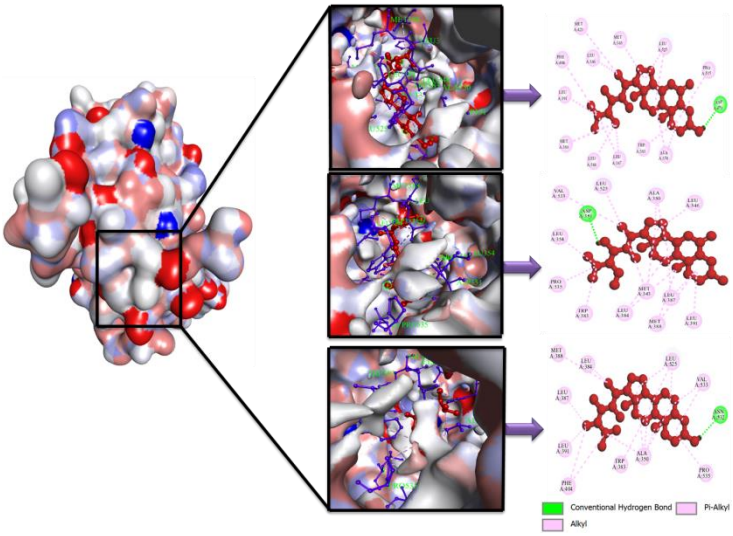


Figure 13. 2D and 3D presentation of ligands teasterone, 28-Homoteasterone, 6-Deoxoteasterone and estrogen receptor interactions.

Likewise, the binding studies performed between the tamoxifen and estrogen receptor, indicates interacting with amino acid residues ASP351, VAL534 via hydrogen bond and with amino acid residues GLU353, LEU354, TRP383, LEU384, MET388, MET421, ILE424, PHE425, GLY521, HIS524, VAL533, PRO535 via Van der waals interaction and LEU346, LEU349, ALA350, LEU387, PHE404, LEU525 residues via Alkyl/π-Alkyl interactions and amino acid residue LEU525 form π-sigma interaction (Table 6 and Fig 17).

Table 6. Conventional drug molecules interaction with specific proteins.

Proteins	Compounds	G-Score kcal/mol	Van der waals	Hydrogen bond	Alkyl/π-Alkyl	π-sigma
HMGCoA reductase (1HW9)	**Simvastatin**	-9.29	GLY656, MET657, ASN658, CYS691, GLY765, GLN766, ASP767, GLY803, THR804, GLY807, THR809.	CYS526, MET655, GLY808.	ALA654.	-
17βhydroxysteroid dehydrogenase (1FDS)	**Abiraterone**	-7.68	GLY145, GLU163, GLY164, ARG252, PHE254, ARG264, ARG266.	PHE160	LEU146, LEU260, LEU267.	LEU263
Aromatase (5JKV)	**Letrozole**	-7.93	ILE132, ILE133, ARG145, LEU152, MET303, ALA306, VAL373, PHE430, GLY431, GLY439	ARGY115, TRP141	ARG435	CYS437
Androgen receptor (1E3G)	**Flutamide**	-1.83	THR739, MET742, GLY743, LEU744, LEU812, VAL866.	SER740, SER814, GLN867.	LEU811, ILE815, ALA870,ARG871 HIS874, ILE906, PRO913.	-
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

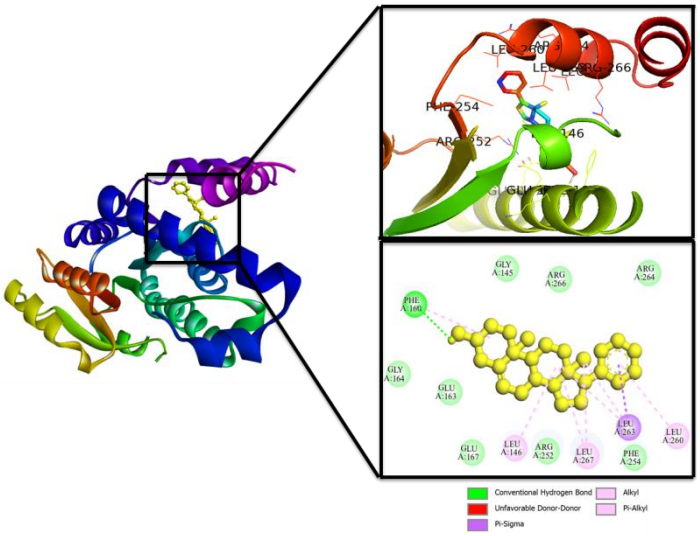


Figure 14. 2D and 3D presentation of abiraterone and 17βHSD interaction.

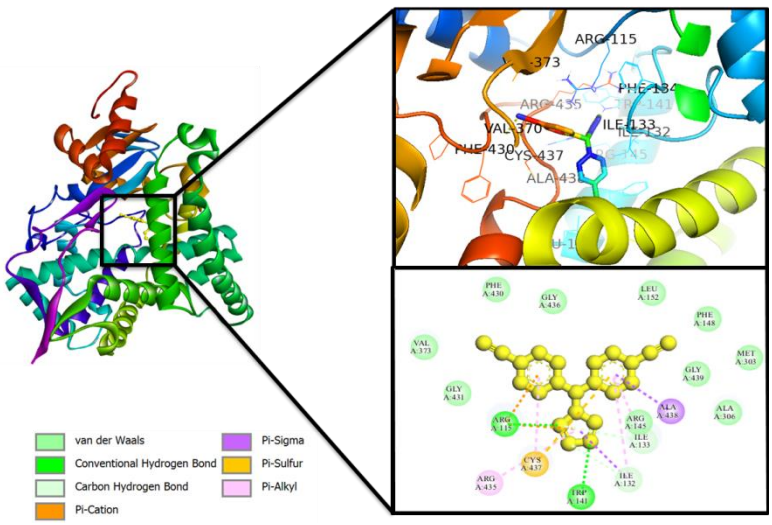


Figure 15. Shows 2D and 3D presentation of letrozole and aromatase enzyme interaction

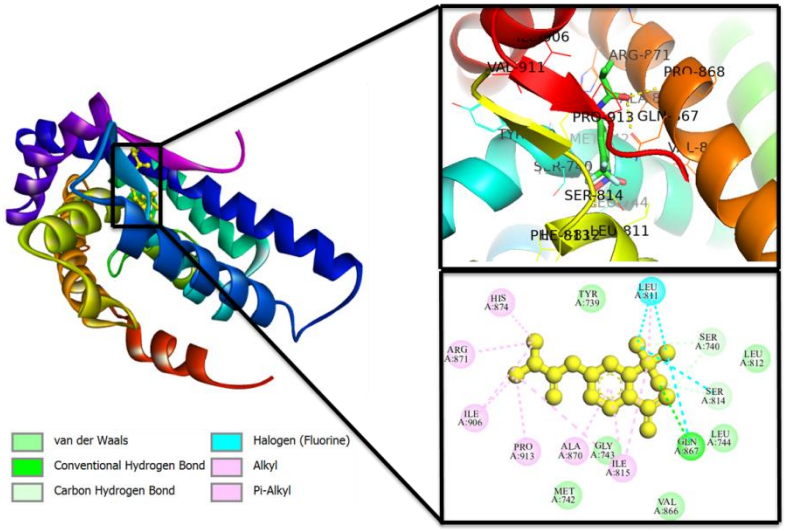


Figure 16. Shows 2D and 3D presentation of flutamide and androgen receptor interaction.

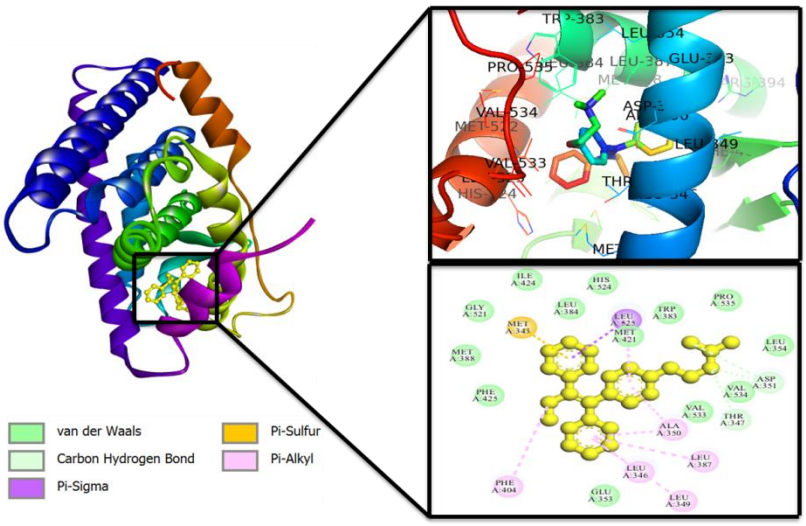


Figure 17. Shows 2D and 3D presentation of tamoxifen and estrogen receptor interaction.

Discussion

Current In Silico analysis targeted daily intake of dietary oxysterol influences on women health. Bearing in mind the biological effectiveness of dietary phyto oxysterols are known, however, studies on role in ovarian sterioidogenesis are inadequate. Phyto oxysterol such as dolicholide, 2-Deoxy-25-Methyldolichosterone, typhasterol, teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone, 6-Deoxo-28-nortyphasterol are ubiquitous in nature made foundations to use target based research in health and disease (1). Present study employing with phyto oxysterols effects on ovarian sterioidogenesis not reported. As PCOS is well known that up regulating ovarian estrogen and testosterone levels, use of In Silico aided method a valuable tool for evaluating the oxysterol inhibitory potential on ovarian sterioidogenesis in PCOS subject (7).

Theca cells are site of androgens synthesis and these androgens transported into granulosa cells, where further androgens are converted into estrogen by aromatase catalytic reaction. The ovarian steroidogenesis in theca and granulosa cells is subjected to cholesterol uptake from HDL, LDL and internal de novo cholesterol synthesis under regulative of LH. Cholesterol synthesis is facilitation by HMGCoA reductase enzyme catalyzing rate limiting step of cholesterol synthesis in tissues and inhibiting HMGCoA reductase an ideal target to down regulate ovarian cholesterol transport and synthesis in theca cells (8). Phyto oxysterols dolicholide, 2-Deoxy-25-Methyldolichosterone, typhasterol, teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone, 6-Deoxo-28-nortyphasterol shows high binding affinity towards HMGCoA reductase compared to simvastatin standard drug molecules highlights their ideal inhibitor of HMGCoA reductase (1).

Further, androstenedione to testosterone conversation step under catalytic action of 17βHSD with presence of cofactor NADPH in granulosa cell. In the PCOS increased testosterone level leads to polycystic ovary, anovulation, irregular menstrual cycle, which can be increased catalytic function of 17βHSD. However, the inhibition of 17βHSD is an ideal beneficial management of PCOS complications. Current study noted that valuable insights among phyto oxysterol and 17βHSD. The phyto oxysterol dolicholide, 2-Deoxy-25-Methyldolichosterone, typhasterol, teasterone, 28-Homoteasterone, 28-Nortyphasterol, 6-Deoxoteasterone, 6-Deoxo-28-nortyphasterol shown high binding affinity with 17βHSD compared to abiraterone standard molecules highlights their ideal inhibitor of 17βHSD in PCOS (9).

Phyto oxysterol shows that inhibitory effects of ovarian sterioidogenesis and androgen receptor, estrogen receptor transactivation. Depiction resemblance to endogenous oxycholesterol regulation in key steroidogenesis pathways in ovary, estrogen and testosterone mediated physiological effects in PCOS. The conversion of HMGCoA to cholesterol and cholesterol to pregnenolone to androstenedione into testosterone than estradiol formed in ovarian cells. Further, testosterone and estrogen homeostasis is behind the feedback regulation of GnRH, LH and FSH signaling mediators (11). In PCOS increased level of testosterone and estrogen resulted to amenorrhoea or oligomenorrhoea and anovulation due to that pathophysiological effect of estrogen. The HMGCoA reductase and aromatase catalytic inhibitor is modulating estrogen level resulted to ovulation take places in PCOS subject (5,6).

Aromatase enzyme comes under the cytochrome p450 family, secreted by granulosa cell of ovary and catalyzes the conversion of testosterone to estrogen irreversibly reaction. This catalytic reaction is the final and rate limiting step in the ovarian estrogen synthesis. 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 (-9.85 kcal/mol), anastrozole (-9.60 kcal/mol) and letrozole (-7.93 kcal/mol) highlights their promising inhibitors for the aromatase enzyme (table). However, daily intake of brassinosteroids may be down regulate estrogen level resulted to preventing the PCOS effects on ovulation (10).

Androgen receptor mediated signaling mechanism in PCOS to generation of altered phenotype traits are down regulation of follicle development leading to multiple small cysts in ovary resulted to anovulation and hirsutism, acne, alopecia in peripheral tissue (6). Hence, inhibition of androgen with androgen receptor interactions is idyllic phenomenon to managing PCOS complications. Hence, in the present work the 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 compounds testosterone (-1.11 kcal/mol) and flutamide (-0.83 kcal/mol) (table) to inhibition of androgen receptor potentially (7,10).

Estrogen receptor can be localized in cell membrane, cytoplasm or nuclease. Estrogen inducing the estrogen receptor resulted to genomic or non-genomic effects such as transcription and cell division. In PCOS estrogen receptor inducing estrogen synthesis, causes anovulation. Therefore, targeting estrogen receptor in PCOS is idyllic phenomenon (11). Hence, the compounds castasterone, brassinolide, 24-Epicastasterone, 6-Deoxocastasterone, 3,24-Diepicastasterone, 6-Deoxo-28-norcastasterone shown highest binding affinities towards estrogen receptor compared to reference compounds tamoxifen (-11.03 kcal/mol) and estradiol (-9.69 kcal/mol) (table) highlights their promising inhibitors for the estrogen receptor in PCOS (7,12).

Ovarian sterioidogenesis rate limiting steps in testosterone and estrogen 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 brassinosteroids. The significant binding avidity between the testosterone, estrogen and brassinosteroid on enzyme catalytic side, suggestive a modulatory effect by brassinosteroids on ovarian sterioidogenesis in human cell. In the present investigation highlights the multifactorial effects of

brassinosteroids in its capability to down regulating testosterone and estrogen levels and inhibition of androgen and estrogen receptors in PCOS subject. Further, In Vivo studies are needed to understanding the possible influences contributing brassinosteroids to ovarian sterioidogenesis in normal and PCOS subjects.

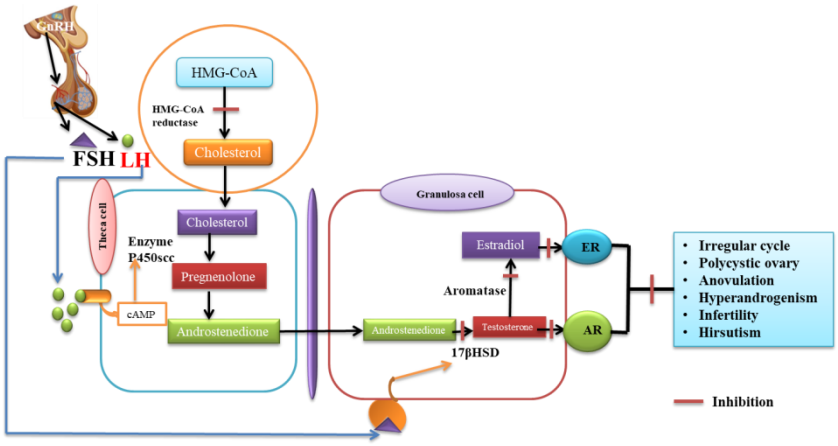


Figure 18. Shows proposed action of phyto oxysterol in PCOS.

Conclusion

The present In Silico study suggests that BS is an effective alternative to potentially inhibit ovarian sterioidogenesis at multiple levels. Present study identified 6α-Hydroxycastasterone, castasterone, 28-Homocastasterone, 6-Deoxocastasterone, 24-Epibrassinolide, 6-Deoxo-28-norcastasterone, brassinolide, 28-Norbrassinolide, 3,24-Diepicastasterone, and 28-Homobrassinolide as potential modulator of ovarian sterioidogenesis through 17βHSD, aromatase, androgen and estrogen receptors inhibitors. Among them 6-Deoxo-28-norcastasterone, castasterone, 24-Epibrassinolide, 6-Deoxocastasterone, 3,24-Diepicastasterone, 28-Norbrassinolide and brassinolide exhibited superior putative ovarian sterioidogenesis inhibitor. These results suggestive novel phyto molecules based managing PCOS. Remarkably, brassinosteroids shown better binding avidity with 17βHSD, aromatase, androgen and estrogen receptors than conventional drug molecules. The outcome of our In Silico data may be the basis for in vitro and in vivo studies against PCOS.

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