Protein modelling, identification of de novo chemical inhibitor and QSAR studies on PCOS (Polycystic Ovary Syndrome) using cheminformatics software and tools

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Abstract
In this *insilico* research project, Polycystic Ovary syndrome analysis was carried out. Protein modeling and drug designing were major steps for delivering the best drug candidates for disease target. The identified protein target gene is (LHCGR-Luteinizing Hormone/Choriogonadotropin Receptor). We deliver the potential chemical inhibitor for LHCGR receptor. We use advanced cheminformatics software and tools to determine the quality of the *De Novo* chemical. QSAR studies clearly elucidate the potential inhibitor for LHCGR protein. The result showed the 2 dimensional structures of the designed *de novo* chemicals displayed by Chemaxon software. These chemicals were introduced to the target protein (LHCGR- luteinizing hormone/choriogonadotropin receptor). The results revealed the designed chemical structure for the *de novo* drug. All the results of the research investigation clearly elucidated that the designed drug candidates is a potential inhibitor for the modeled target protein (LHCGR - luteinizing hormone/choriogonadotropin receptor).

Key word: Protein Modelling, Drug Designing /Docking and QSAR

INTRODUCTION
Polycystic Ovary Syndrome also called as PCOS is an imbalance of the Female reproductive hormones. The ovaries are part of the female reproductive system along with the fallopian tubes, uterus and vagina and contain lifetime supply of eggs. These eggs are immature and are stored in the tiny fluid filled structures called Follicles. Pituitary gland located at the base of the brain, produces the hormones that direct the function of the ovaries. Each month, the pituitary gland secretes Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) into the blood stream. After these hormone reach the ovaries, several hundreds of immature eggs start to mature [1].

As the eggs mature the follicles secrete estrogen, the main female sex hormone. Once the amount of estrogen in the blood reaches certain level, the pituitary sends a surge of Luteinizing Hormone to the ovaries causing the most mature follicles to open and release its egg in a process called Ovulation. The free egg travels through fallopian tube where it waits for fertilization, eventually the remaining immature follicles and eggs dissolve. If the egg is not fertilized, the egg and lining of the uterus are shed during the next menstrual period. If a woman has Polycystic Ovary Syndrome, pituitary gland may release abnormally high amounts of Luteinizing Hormone into the blood stream, disrupting the normal menstrual cycle. As a result, follicles do not mature and ovulation does not occur, which can lead to infertility. Some of the immature follicles do not dissolve and remain as a fluid filled sacs or cysts [2]. In addition, the blood contains high levels of Insulin, a hormone produced by the pancreas. Too much insulin combined with high levels of Luteinizing Hormone can lead to excess production of male hormone called Testosterone in the ovaries. Abnormally high levels of testosterone prevent ovulation which can lead to infertility. High levels of testosterone also cause many of the physical features associated with the polycystic ovary syndrome such as acne and abnormal hair growth [3].

AIMS AND OBJECTIVES
- To find out the potential protein receptor

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(LHCGR) present in polycystic ovary syndrome using literature databases.
- To retrieve protein sequences for polycystic ovary syndrome and to access the protein function.
- To predict three-dimensional structure of the proteins.
- To visualize the protein models.
- To validate the chemical structure of the protein.
- To select the suitable drugs.
- To design the de novo drug.
- To validate the ligands and predict the toxicity using QSAR studies.
- To perform comparative docking of the designed de novo ligands with the target protein receptor LHCGR).

METHODOLOGY

Bioinformatics

Literature studies
- The complete molecular genetic information related to Polycystic Ovary Syndrome was studied using the human genetic disease databases, OMIM (Online Mendelian Inheritance in Man). Other literature medical databases like PUBMED and PUBMED CENTRAL were also used.

Target identification
- Based on literature information the potential gene involved in polycystic ovary syndrome was identified. The target gene (LHCGR-Luteinizing Hormone/Choriogonadotropin Receptor) which is directly involved in PCO syndrome was selected for research.

Molecular modeling
- The identified target gene (LHCGR-Luteinizing Hormone/Choriogonadotropin Receptor) coded protein sequence was retrieved from NCBI in FASTA format. The retrieved protein sequence was applied into CPH 3.0 model server in order to predict the 3 Dimensional structure of the protein sequence.

3D structure visualizations:
- The modeled protein structure was viewed with help of advanced visualization tools like DISCOVERY STUDIO, MOLSOFT, MOLEGR0 MOLECULAR VIEWER and RIBBON.

Cheminformatics

Drug designing
- The existing chemical compounds for polycystic ovary syndrome were collected from NCBI PUBCHEM compound databases and were combined with other chemical agents in order to increase the efficiency of the existing molecules using CHEMAXON software.

2D to 3D structure prediction
- The designed 2 dimensional de novo drug candidates were converted into 3 dimensional structures using online smiles translator and viewed with the help of DISCOVERY STUDIO SOFTWARE.

chemaxon’s chemicalize.org
- Chemicalize.org is a public website which extracts and displays chemical structures from users browsing of web pages and submitting other documents to the site. The service builds a database of all identified structures, which enables structure (and other) searching of gathered content.

QSAR(Quantitative Structure Activity Relationships prediction)
- The designed de novo drugs were validated using an advanced QSAR - Quantitative Structure Activity Relationships prediction software like (VegaNIC-1.0.6-binaries) in order to identify the overall quality of the designed chemical molecules.

Drug validation
- The identified chemicals were applied into CHEMAXON (Marvin Sketch) software in order to find out the interactions of (Metformin+Mefemanic acid, Metformin +Phenobarbitone, Cyproterone acetate +Lithium, Levonorgestrel+ Aspirin, Estradiol+Meformin HCl and Minoxidil +Flutamide) present in the designed de novo molecules.

Molecular drug docking
- The designed chemical molecules were docked with the modeled protein target (LHCGRLuteinizing Hormone/ Choriogona dotropin Receptor) using an automatic molecular drug docking server, PATCHDOCK. The results were validated based on the atomic contact energy values and finally the drug and protein binding regions are viewed in DISCOVERY STUDIO SOFTWARE.
RESULTS
The studies on Polycystic Ovary Syndrome (PCOS) are made through literature databases such as PubMed Central and PubMed. The gene was identified using NCBI-gene databases. The gene LHCGR (Luteinizing Hormone/Choriogonadotropin Receptor) was selected for Polycystic Ovary Syndrome.

PROTEIN MODELING
The amino acids sequence of the identified protein target (LHCGR–luteinizing hormone/choriogonadotropin receptor) showed 699 amino acids. The retrieved sequences were converted into 3D structure using CPH 3.0 Model server. The identified protein target gene (LHCGR-Luteinizing Hormone/Choriogonadotropin Receptor) coded protein sequence was retrieved from NCBI in FASTA format. The three dimensional structure prediction of selected disease target Luteinizing Hormone/Choriogonadotropin Receptor was modelled through advanced automated fold recognition modelling server. Determining the structure and function of a novel protein is an important step. The retrieved protein sequence was applied into CPH 3.0 model server in order to predict the 3 dimensional structure of the protein sequence. The CPH 3.0 model server was used for protein modelling. The identified target protein (LHGR - luteinizing hormone/choriogonadotropin receptor) sequence was applied into CPH 3.0 model server and the results showed the best template based modelling protein structure. The structure was downloaded and viewed with the help of advanced visualization tools like Discovery Studio Software, Molsoft, Molegro Molecular Viewer and Ribbon. This protocol provides a guide to interpreting the output of structure prediction in general and one such tool in particular, the protein homology/analogy recognition engine. This was viewed through molecular visualization softwares (Discovery studio, Rasmol, Molsoft and Molegro molecular viewer and Ribbon).

Drug Designing
In drug designing, using NCBI- PUBCHEM compound databases, the following existing drugs (Metformin, Cyproterone acetate, Levonorgestrel, Mefformin Hydrochloride and Minoxidil) and the selected agents (Mefemanic acid, Phenobarbitone, Lithium, Aspirin, Estradiol and Flutamide) were chosen and combined with the help of an advanced Cheminformatics software called Chemaxon. It includes tools for visualization and drawing of molecules, chemical database searching management and for drug discovery. The results showed the 2 dimensional structure of the designed de novo chemicals displayed by Chemaxon software. These chemicals were introduced with target protein (LHGR - luteinizing hormone/choriogonadotropin receptor). The results revealed the designed chemical structure for the de novo drugs.

Drug Validation
Chemaxon chemicalize.org
Chemaxon’s Chemicalize.org was used for the complete chemical structure validation of the de novo drugs such as Molecule, Name and identifier, Elemental analysis, pKa, Isoelectric point, LogP, LogD, Charge, Polarizability, Orbital electronegativity, Tautomerization and Topology analysis.

QSAR (Quantitative Structure Activity Relationships prediction)
In QSAR studies VegaNIC-1.0.6-binaries software was applied for the designed chemicals to interact with human database references. The software represented the advanced molecular interaction of the de novo drug. Validation of de novo drugs by using QSAR validation, showed the correlation between chemical structure and associated biological activity, with the ultimate goal of predicting the activity of untested chemicals based on structurally related compounds with known activity. They were used to predict the following prediction models such as Mutagenicity model, Mutagenicity SarPy model, Carcinogenicity model and Skin sensitization model, which are very important tools to predict about each and every de novo drug. The QSAR results of de novo drugs were shown. These prediction summaries played an important role in toxicity validation of de novo drugs. The QSAR validation of de novo drugs were summarized in Table 1.

Molecular docking
In Molecular drug studies, PatchDock server was used. The modelled target protein(LHGR - luteinizing hormone/choriogonadotropin receptor) and the designed drug candidates were docked.
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PatchDock employs this technique. Given two molecules, their surfaces are divided into patches according to the surface shape. These patches correspond to patterns that visually distinguish between puzzle pieces. Once the patches are identified, they can be superimposed using shape matching algorithms. The best Protein-ligand interaction was found to be in the de novo drug (Levonorgestrel + Aspirin with the value of -512.74) with LHCG (Luteinizing Hormone/Choriogonadotropin Receptor) (Table-2).

The Table 2 showed patch dock results of protein ligand complex of the de novo drugs and drug interactions.

Figures 1-6 showed the protein-ligand interaction studies of Polycystic Ovary Syndrome (PCOS) protein with de novo drugs.

Figures 1 and 2 showed the interaction of protein LHCGR and de novo ligands Metformin with Mefenamic acid and Metformin with Phenobarbitone.

Figures 3 and 4 showed the interaction of protein LHCGR and de novo ligands Cyproterone acetate with Lithium and Levonorgesterol with Aspirin.

Figures 5 and 6 showed the interaction of protein LHCGR and de novo ligands Metformin Hydrochloride with Estradiol and Minoxidil with Flutamide.

Table 1. QSAR (Quantitative Structure Activity Relationships rediction) VALIDATION

<table>
<thead>
<tr>
<th>S. No</th>
<th>De Novo Drugs</th>
<th>(Mutagenicity Model)</th>
<th>(Mutagenicity SarPy Model)</th>
<th>(Carcinogenicity)</th>
<th>(Skin Sensitisation Model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Metformin with Mefenamic acid</td>
<td>Reliability</td>
<td>NON-Reliability</td>
<td>NON-Carcinogen</td>
<td>Sensitizer</td>
</tr>
<tr>
<td>2.</td>
<td>Metformin with Phenobarbitone</td>
<td>Reliability</td>
<td>Reliability</td>
<td>NON-Carcinogen</td>
<td>NON- Sensitizer</td>
</tr>
<tr>
<td>3.</td>
<td>Cyproterone acetate with Lithium</td>
<td>NON-Reliability</td>
<td>Reliability</td>
<td>NON-Carcinogen</td>
<td>NON- Sensitizer</td>
</tr>
<tr>
<td>4.</td>
<td>Levonorgesterol with Aspirin</td>
<td>NON-Reliability</td>
<td>NON-Reliability</td>
<td>NON-Carcinogen</td>
<td>NON- Sensitizer</td>
</tr>
<tr>
<td>5.</td>
<td>Metformin HCl with Estradiol</td>
<td>Reliability</td>
<td>NON- Reliability</td>
<td>NON-Carcinogen</td>
<td>Sensitizer</td>
</tr>
<tr>
<td>6.</td>
<td>Minoxidil with Flutamide</td>
<td>Reliability</td>
<td>Reliability</td>
<td>NON-Carcinogen</td>
<td>NON- Sensitizer</td>
</tr>
</tbody>
</table>

Reliability - Dependable, stability; NON-Reliability - Undependable; Sensitizer –Activator; Non- Sensitizer – Inhibitor; NON-Carcinogen -Benignancy

**Fig: 1. 3 Dimentional structure of de novo ligand (Metformin+Mefenamic acid) with protein (Luteinizing hormone/Choriogonadotropin receptor)**

Blue – De Novo drug (C₃₁H₄₂N₂O₇) and protein (LHGR) trace model – Secondary structure view
Fig 2. 3 dimensional structure of de novo ligand (metformin+phenobarbitone) with protein (luteinizing hormone/choriogonadotropin receptor)

Blue – *De Novo* drug (C₁₅H₁₉N₇O₃Na) and protein (LHCGR) trace model – secondary structure view

Fig 3. 3 Dimensional Structure Of *De Novo* Ligand (Cyproterone Acetate+Lithium) With Protein (Luteinizing Hormone/Choriogonadotropin Receptor)

Blue – *De Novo* drug (C₂₄H₂₈ClLiO₄) and protein (LHCGR) trace model – secondary structure view

Fig 4. 3 Dimensional Structure Of *De Novo* Ligand (Levonorgesterel+Aspirin) With Protein (Luteinizing Hormone/Choriogonadotropin Receptor)

Blue – *De Novo* drug (C₃₀H₃₄O₆) and protein (LHCGR) trace model – secondary structure view
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Fig. 5. 3 dimensional structure of de novo ligand (Metformin hydrochloride+ Estradiol) with protein (Luteinizing hormone/Choriogonadotropin receptor)

![3D structure of Metformin hydrochloride+ Estradiol with LHCGR](image)

Blue – De Novo drug (C21H30N2O2Cl) and protein (LHCGR) trace model – secondary structure view

Fig 6. 3 Dimentional Structure of De Novo Ligand (Minoxidil+Flutamide) With Protein (Luteinizing Hormone/Choriogonadotropin Receptor)

![3D structure of Minoxidil+Flutamide with LHCGR](image)

Blue – De Novo drug (C20H26N2O4F3) and protein (LHCGR) trace model – secondary structure view

Table 2. Receptor –drug interaction: molecular drug docking patch dock results of protein ligand complex (de novo drug)

<table>
<thead>
<tr>
<th>Protein Target</th>
<th>Metformin+ Mefemanic Acid</th>
<th>Metformin + Phenobarb itone</th>
<th>Cyproter one acetate+ Lithium</th>
<th>Levonorg estrel+ Aspirin</th>
<th>Estradiol+ Meformin HCl</th>
<th>Minoxidil+Flutamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>(LHCGR) LUTEINIZING HORMONE/CH ORIOGONADO TROPIN RECEPTOR</td>
<td>-389.02</td>
<td>-408.28</td>
<td>-396.15</td>
<td>-512.74</td>
<td>-142.04</td>
<td>-110.32</td>
</tr>
</tbody>
</table>

The above table shows binding affinity of protein and De Novo ligands (Levonorgestrel+Aspirin shows higher binding affinity (-512.74))
A ligand is a substance that forms a complex with a bio-molecule to serve a biological purpose. In a narrower sense, it is a triggering molecule, binding to a site on a target protein. The binding occurs by intermolecular forces, such as ionic bonds, Vander Waals forces [4].

The interaction of most binding ligands with their binding sites can be characterized in terms of binding affinity. In general, high affinity ligand binding results from inter molecular forces between the ligand at its receptor while low affinity ligand binding involves less intermolecular force between the ligand and its receptor. High affinity binding of ligands to receptors is often physiologically important when some of the binding energy can be used to cause a conformational change in the receptor, resulting in altered behaviour of an associated ion channel or enzyme.

Binding affinity data alone does not determine the overall potency of a drug. Potency is a result of the complex interplay of both the binding affinity and ligand efficacy. Ligand efficacy refers to the ability of the ligand to produce a biological response upon binding to the target receptor and the quantitative magnitude of the response.

Docking is the most commonly used method in the field of drug designing. Docking is frequently used to predict the binding orientation of small molecule drug to their target protein in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme or hormone. If the protein is a receptor, ligand binding may result in agonism or antagonism [5].

In women with PCOS, the ovary doesn’t make all of the hormones it needs for an egg to fully mature. The follicles may start to grow and build up fluid but ovulation does not occur. Instead, some follicles may remain as cysts. For these reasons, ovulation does not occur and the hormone progesterone is not made. Without progesterone, a woman’s menstrual cycle is irregular or absent. In addition, the ovaries make male hormones, which also prevent ovulation.

PCOS affects many systems in the body. So, many symptoms may persist even though ovarian function and hormone levels change as a woman nears menopause. For instance, excessive hair growth continues, and male-pattern baldness or thinning hair gets worse after menopause. Also, the risks of complications (health problems) from PCOS, such as heart attack, stroke, and diabetes, increase as a woman gets older.

Women with PCOS are also at risk for endometrial cancer. Irregular menstrual periods and the lack of ovulation cause women to produce the hormone estrogen, but not the hormone progesterone. Progesterone causes the endometrium to shed each month as menstrual period. Without progesterone, the endometrium becomes thick, which can cause heavy or irregular bleeding. Over time, this can lead to endometrial hyperplasia, when the lining grows too much, leading to cancer. There is a tendency for polycystic ovary syndrome to be more common in people who have a close relative with the condition. The fact that the condition can run in families suggests that there is a genetic component to the disease [6].

There seems to be evidence linking polycystic ovary syndrome with higher than normal levels of a hormone known as insulin. Insulin is a hormone that controls blood sugar levels. A lot of women with polycystic ovary syndrome have a problem known as insulin resistance. People with insulin resistance need higher levels of insulin to be released to control their blood sugar. This means their body is exposed to higher amounts of insulin than other people. One of the effects of these high levels of insulin is that the ovaries make too much testosterone. The high levels of testosterone are responsible for some of the symptoms of polycystic ovary syndrome, including acne and excess hair.

Increase in weight can worsen the problem. This is because having excess body fat also makes insulin resistance worse, and so the levels of insulin in the body are increased even more. Another hormone known as luteinising hormone is also raised in some women with polycystic ovary syndrome. Luteinising hormone also increases levels of testosterone in the body.

There is no cure for PCOS, it needs to be managed to prevent problems. Treatment goals are based on the symptoms, whether or not a woman wants to become pregnant, and lowering the chances of getting heart disease and diabetes. Many women will need a combination of treatments to meet these goals. Most PCOS patients are inherently insulin resistant, with obesity seen in many, only adding to this problem. A substantial proportion of
PCOS patients have abnormalities on the oral glucose tolerance testing at the time of diagnosis. In 2000, a multicentric study involving seven urban cities (Chennai, Bangalore, Hyderabad, Mumbai, Calcutta, and New Delhi) in India among the age group of 20-40 years indicated that the prevalence rate of obesity was 31%. It has been reported a 37.5% prevalence rate of obesity in women with PCOS exists [7]. The prevalence of glucose intolerance is significantly higher in PCOS women than in concurrently studied age ethnicity and weight-matched ovulatory control women [8].

The increased daytime blood pressures in women with PCOS and greater increases in pulse rate from night to daytime recordings persisted after adjusting for BMI, body fat distribution, and insulin resistance. Thus, women with PCOS have an increased prevalence of labile blood pressure, which may indicate a prehypertensive state, adding a further risk factor for cardiovascular disease [9].

It is difficult to be certain on clinical grounds that ovulation is occurring. The only absolute proof of ovulation is pregnancy, but there are observation and routine investigations which may help in assessment. A regular cycle and dysmenorrhea suggest, but do not prove, that ovulation is occurring. Conversely the woman with amenorrhea is unlikely to be ovulating, although should this occur and fertilization be successful, it can cause considerable confusion in calculating the length of the gestation. Sometimes women notice lower abdominal pain for a brief period at ovulation. The body temperature dips during the follicular phase, and then rises at the time of ovulation, both in those who want to conceive and in those who do not.

A plasma progesterone level of 10 nmol/l or more, seven days before menstruation (i.e. seven days after ovulation) indicates the formation of a corpus luteum, although the range in the luteal phase is 5-60nmol/l. Urinary pregnanediol is also increased, this being an end product of progesterone metabolism which is easily measured. The plasma progesterone level is nevertheless the most reliable test.

A vaginal smear is easily obtained even in a virgin and on histological examination may reveal characteristic cellular changes if there is sufficient progesterone. In addition, the following vaginal smear characteristics may be of value. Visualization of the corpus luteum by laparoscopy is good evidence for ovulation having occurred. The medicine metformin is used to treat type 2 diabetes. Metformin was widely used drug for PCOD. Metformin affects the way insulin controls blood glucose (sugar) and lowers testosterone production. It slows the growth of abnormal hair and, after a few months of use, may help ovulation to return. Recent research has shown metformin to have other positive effects, such as decreased body mass and improved cholesterol levels. Metformin will not cause a person to become diabetic.

There are now large numbers of studies published on the effect of Metformin in a dose of 1500-2550 mg/day in women with PCOS. The vast majority of these studies have demonstrated a significant improvement in insulin concentrations, insulin sensitivity and serum androgen concentrations accompanied by decreased Luteinizing Hormone [10]. The restoration of regular menstrual cycles by Metformin has been reported in large majority of patients and ovulation has occurred in 78%-96% of patients [11]. No adverse reactions were recorded for the use of Metformin. So it is safe drug and has no reports on teratogenicity. Metformin reduced the risk of ovarian hyperstimulation syndrome.

It was proved that almost all patients who have taken Metformin have attained follicular maturation and regularization of menstruation. It has recently been used to treat hirsutism, in doses from 500mg, 3 times per day. Metformin in combination with Mefenamic acid was prepared. Mefenamic acid is given for patients having intense abdominal pain during menstruation [12].

Mefenamic acid helps to reduce inflammation and to reduce pain. Mefenamic acid works by blocking the production of some of the body chemicals that cause inflammation, pain, stiffness, tenderness, swelling and increased temperature. By reducing inflammation in conditions affecting muscles and joints Mefenamic acid helps to improve movement. Mefenamic acid can take a few weeks to help improve inflammation but can start to relieve pain after the first few doses. This medicine will normally be prescribed at the lowest possible dose for the shortest time to reduce the chance of side-effects [13].

Non-steroidal anti-inflammatory drugs (NSAIDs) are highly effective in the reduction of menstrual bleeding and pain. Mefenamic acid have been shown to reduce blood loss by 30 - 50%, and only need to be taken during the menstrual period [14]. This research was conducted to compare their efficacy in reducing heavy bleeding. Comparing
oral contraceptive pills. Mefenamic acid showed no significant differences in the amount of menstrual bleeding between groups. Another positive finding was that the majority of the women in the intervention groups were satisfied with the drugs used for treating their problem [15].

Reproductive endocrine dysfunction in women with epilepsy is an important issue, and in recent years there is growing evidence to support the effect on sex hormones of both epilepsy per se and various antiepileptic drugs (AEDs). Reproductive endocrine dysfunction is more common among women with epilepsy than in the healthy population [16][17]. It manifests itself as menstrual disorder, hirsutism, and polycystic changes in the ovaries [18]. However, it is difficult to determine whether hormonal abnormalities are due to epilepsy-related hypothalamic–pituitary axis (HPA) dysfunction or to side effects of antiepileptic drugs (AEDs). The incidence of PCO and PCOS appears to be more common among women with epilepsy than among women without epilepsy. The use of liver enzyme-inducing antiepileptic drugs such as Phenytoin, Carbamazepine can increase serum sex hormone-binding globulin concentrations, leading to diminished bioactivity of testosterone. There have been unfortunately only limited studies of prevalence of PCOS and ethnic influences.

PCOS may be more common among African Americans than Caucasians, based on a prevalence study among 400 US women [19]. In regards to ethnic variation, Asian with PCOS may have similar circulating levels of androgens as other ethnic groups, but little hirsutism [20]. This may be due to differing peripheral tissue specific expression of genes that modify androgen. Insulin resistance appears more common among certain ethnic groups, for instance those of Latino Caribbean [21] or south Asian origin [22][23] compared to Caucasians. These ethnic differences in phenotype suggest a link to inherited alleles that influence the expression of traits.

Sexual dysfunction is a common issue with 40-45 percent of women in general, and the likelihood of these concerns increases with age and the presence of health issues such as high blood pressure and diabetes.2 This means women with PCOS can experience a higher incidence of poor sexual health due to certain appearance-related symptoms and the increased risk of those physical conditions (diabetes and poor cardiovascular health) that cause sexual dysfunction. Women with PCOS should never hesitate to discuss sexual health concerns with their doctor, and they should follow recommended strategies to address PCOS and factors such as Insulin Resistance to minimize symptoms and improve overall quality of life.

Cyproterone acetate has been widely used in PCOS. It is effective in treatment of both hirsutism and acne [24]. It acts mainly by competitively binding the androgen receptor. In mild to moderate cases, cyproterone acetate in a dose of 2 mg/day combined with 35 μg of ethinyl estradiol generally improves the symptoms [25]. PCOS patients often contact a health care provider during their teenage years for unpredictable uterine bleeding. Use of oral contraceptive pills such as cyproterone acetate and levonorgestrel these patients results in regular withdrawal bleeding in addition to improvement in hyperandrogenism. This is a progestogen and anti-androgen. It is highly effective in hirsutism but some women experience fluid retention and other side effects.

For women who don’t want to get pregnant, birth control pills can control menstrual cycles, reduce male hormone levels and help to clear acne. The oral contraceptive pills have the ability to address many of the goals of reproductive-aged women with PCOS not seeking pregnancy. They ameliorate hyperandrogenic skin manifestations, regulate menstrual cycles thereby protect from the risk of endometrial carcinoma, and provide effective and safe contraception [26].

Psychiatric disorders should be considered in PCO women. Fasting blood sugar (FBS) and LH levels were significantly higher in PCO women than other group. These findings can be indicative of metabolic relationship between PCO and mood disorders. On the other hand, Rasgon [27] found that menstrual disturbances in women with bipolar disorder are common. Lithium used to reduce the severity of the bipolar disorder and frequency of mania. It may also help to relieve depressive episodes. It helps to strengthen the nerve cell connections in the brain regions that are involved in regulating mood, thinking and behaviour. Bipolar disorders were higher in PCOS group.

Aspirin has become the extremely popular anti-pyretic, anti-inflammatory and analgesic agent. The crucial discovery of the biochemical mechanism of the action of aspirin was made by John Vane in 1971 [28]. He observed that aspirin blocked the enzymatic activity of cyclooxygenase (COX), a key
enzyme leading to the production of pro-inflammatory prostaglandins from arachidonic acid. He thus demonstrated the reasons for the anti-inflammatory, analgesic, antipyretic and toxic effects of the most widely used remedy of all time. Beneficial effect of metformin doesn’t seem to be related to reduce androgen levels, since a survey says that Metformin treatment in pregnant women with PCOS reduced pregnancy complication without influencing maternal androgen levels, but seems to reduce uterine artery impedance between 12-19 weeks of gestation. In this manner, administered low dose aspirin in early pregnancy of women at risk of preeclampsia with abnormal uterine artery, Doppler may reduce risk of preeclampsia [29]. Low dose aspirin appears to be useful in high risk women of developing preeclampsia and some clinicians recommend low dose aspirin for women at moderate or high risk for preeclampsia. No adverse maternal or fetal effects related to low dose aspirin have been reported. It has been reported that aspirin and metformin improved utero placental circulation and reduced pregnancy complications [30].

A recent uncontrolled pilot study on PCOS patients reported that treatment with estradiol (30 µg) resulted in decrease in androgen levels after 3 months and improvement in acne without a significant change in hirsutism. Estradiol stimulate the hepatic production of sex hormone binding globulin, resulting in increased binding of testosterone and thus reducing the level of active free testosterone.

Women with PCOS have four times the rate of hypertension as those who do not have PCOS. Insulin resistance and hyperinsulinemia raise blood pressure. High levels of insulin correlate with low sodium in the urine leading to water retention leading to an increase in blood pressure. Elevated insulin levels raise blood pressure by stimulating the muscular layer of arterial walls to thicken and stiffen. This causes the heart to work harder to pump blood through the circulatory system.

Minoxidil is a vasodilatant and was originally used as an oral medication for high blood pressure. The drug has no influence on hormone levels. Although minoxidil stimulates hair growth, in fact, this does nothing to the underlying cause of androgenic alopecia.

Flutamide 250mg, 12-hourly, is a non-hormonal anti-androgen and is useful in women in whom previous treatments have been unsuccessful. Liver function needs to be monitored during use. Flutamide reduced visceral fat, androgen levels and lipid profile after 12 months and improved glucose tolerance value. Flutamide reduces androgen levels without improvement of insulin resistance in non-obese patients. Gambineri [31] studied 40 obese PCOS patients during 6 months and showed that hypocaloric diet with flutamide in combination had a significant effect on body lipid distribution, androgen levels, hirsutism and menses [32]. Flutamide reduced triglycerides more than other medicines except hypocaloric diet. This finding is not in agreement with Gambineri [33]’s findings which showed none of the active treatment groups differed in changes of lipid concentrations after 6 months of treatment, whereas they observed a greater reduction in LDL cholesterol. Medicines called anti-androgens may reduce hair growth and clear acne. Anti-androgens are often combined with birth control pills.

In view of the potential for and actual presence of numerous cardiovascular and metabolic risk factors in most women with PCOS, the role of the clinical endocrinologist is essential in the following:

- Early recognition of the syndrome.
- Lifestyle modification, with emphasis on the need for controlled eating patterns and regular aerobic exercise. Encouragement should be offered by an empathic physician, who will monitor the patient carefully during the course of treatment.
- Measurement of glucose (and possibly insulin levels). An oral glucose challenge may be considered, particularly in obese women with PCOS and those with a family history of T2DM.
- Detection and treatment of lipid abnormalities, with dietary measures first and then use of appropriate medications, such as a statin, fibrate, niacin, or ezetimibe (or some combination of these agents), as necessary.
- Careful attention to and treatment of blood pressure abnormalities.

Measurement of atherogenic markers (CRP, fibrinogen, and possibly homocysteine).

Consideration of metformin therapy as the initial intervention in most women with PCOS, particularly in those who are overweight or obese. Metformin improves many metabolic abnormalities in PCOS and may improve menstrual cyclicity and the potential for pregnancy. Of note, metformin has not been approved by the US Food and Drug Administration for use in PCOS, although abundant medical literature supports its efficacy.
The use of a nonandrogenic oral contraceptive agent and an anti-androgen such as spironolactone for the skin manifestations of PCOS. The presence of hair thinning requires the maximal dose of spironolactone in conjunction with an oral contraceptive agent. Ancillary use of electrolysis and laser therapy may also be helpful.

Conventional drug discovery process focuses on known pathological phenomenon and then develops a therapy to combat it. The process of therapy is chemistry based. As there are plethora of new potential therapeutic drug targets that are being discovered, selection and validation of novel molecular targets has become important. It needs to be confirmed that the targets identified will affect an appropriate biological response. The result is the determination of specific receptor targets that must be modulated to alter their activity in some way.

Docking refers to the ability to position a ligand in the active site of a protein and calculate specific binding affinities. The role of drug is to covert the function of the receptor. This approach has been used to predict de novo therapeutic drug to inhibit the potential side effects and to reduce the secretion of luteinizing hormone and androgen in PCOS women.

CONCLUSION
In this Insilico research project, Polycystic Ovary syndrome analysis was carried out. Protein modeling and drug designing were major steps for delivering the best drug candidates for disease target. In this Insilico drug designing studies advanced software tools were used to deliver the drug candidates. The identified modeled protein (LHCGR - luteinizing hormone/choriogonadotropin receptor) and drug candidates (Metformin +Mefemanic acid, Metformin + Phenobarbitone, Cyproterone acetate+Lithium, Levonorgestrel+ Aspirin, Meformin hydrochloride+Estradiol and Minoxidil With Flutamide) were well analyzed and validated using Cheminformatics softwares.

All the results of the research investigation clearly elucidated that the designed drug candidates are potential inhibitors for the modeled target protein (LHCGR - luteinizing hormone/choriogonadotropin receptor).

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