In silico identification of target fetal protein(s) in the development of polycystic ovarian syndrome

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Abstract

Polycystic ovarian syndrome (PCOS) is a multifactorial reproductive disorder mainly affecting ovulating women. Animal studies to date have identified hyperandrogenicity as one of the major causes of PCOS, while estrogen treatment temporarily decreases symptoms. Researchers believe that a high androgen level in a pregnant woman during pregnancy results PCOS-like symptoms in the newborn female baby, which are expressed later during reproductive age. The present work is an in silico analysis of the effect of hyperandrogenicity during fetal ovarian development. An alteration in the level of steroid hormone (androgen, estrogen, progesterone, and testosterone) reportedly affects gonadotropin-releasing hormone (GnRH) secretion and later on the development of ovarian follicles. Nuclear receptors such as DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia congenital critical region on the X chromosome, gene 1) and steroidogenic factor-1 (SF-1) also affect the level of various sex hormones and ovary formation. Hence, the study was carried out to evaluate the effect of these steroid hormones on the promoter region of KISS1 gene and DAX1 gene apart from the effect of these steroid hormones on the binding of kisspeptin (involved in GnRH secretion) and SF1 protein (involved in DAX1 expression modulation). The study involved molecular modeling of promoter regions of KISS1 and DAX1 gene; kisspeptin and SF1 proteins, followed by molecular dockings studies of these promoter regions and proteins against steroid hormone (androgen, estrogen, progesterone, and testosterone), taken as ligand. The study reflected that both the androgen and progesterone show binding over the TATA box of the KISS1 gene, which can be inferred to possibly regulate its expression and affect GnRH secretion to imbalance hypothalamus-pituitary-gonadal axis. This alteration may further cause an abnormal luteinizing hormone: Follicle-stimulating hormone ratio that may result in abnormal steroidogenesis. The molecular docking studies of SF1 protein against DAX1 promoter region were observed to be better than binding when SF1 protein was complexed with studied steroid hormones. The observations lead to the inference that binding of steroid hormones with SF1 protein lowers the expression of DAX1 gene, as the former is essentially required for DAX1 gene expression, which may result in abnormal ovary development in a female fetus as well as abnormal sex steroids level. Thus, it may be concluded that over-secretion of sex steroids is likely to affect female fetus development and hypothalamus-pituitary-gonads axis to trigger PCOS-like symptoms.

1. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder among reproductive women accounting for about 10% of the female population [1,2]. About 84% of PCOS women have irregular menses and 70–80% are infertile, making it a severe disorder among reproductive women. Rotterdam consensus workshop proposed ovarian dysfunction, hyperandrogenism, and polycystic ovaries as a few critical features for identifying PCOS [3,4]. Other PCOS-associated signs include menstrual regulation, obesity, insulin resistance, an elevated luteinizing hormone (LH), and abnormal follicle-stimulating hormone (FSH) level [5,6] are also observed in PCOS women. More than 98% of PCOS women show an imbalanced LH/FSH ratio from its normal 1:1 ratio, whereas most PCOS women show a high LH/FSH ratio [7-9]. LH treated theca cell shows overexpression of cytochrome P450 family 17 subfamily A (CYP17), resulting in the conversion of progesterone to androgen [2]. In women, hyperandrogenism (high androgen level) shows a high risk of developing gestational diabetes, pregnancy-induced hypertension, and preterm birth, resulting in neonatal complications.

During pregnancy, maternal hyperandrogenism is the potential source of hyperandrogenism in developing female fetuses [10,11], which has been proposed as the leading cause of PCOS post-puberty [12]. During pregnancy, hyperandrogenic females’ placenta secretes a low amount of aromatase and a high amount of 3-beta-hydroxysteroid
dehydrogenase (3-ß HSD1) [13], which result in low estrogen and high androgen level in developing fetus. Animal studies in rhesus monkeys and sheep have confirmed many of the characteristic features of PCOS on excess androgen exposure during fetal life [3].

Neuroendocrine abnormalities have also been observed to be involved in PCOS. LH/FSH secretion is mediated by the gonadotropin-releasing hormone (GnRH) pulsatile secretion, where fast GnRH pulse frequency (>1 pulse/h) regulates LH surge and regular pulse frequency (<1–2 pulse/2–3 h) regulates normal LH/FSH secretion. GnRH secretion is regulated by its upstream protein kisspeptin. Various in vivo and in vitro studies revealed that kisspeptin administration stimulates GnRH and LH secretion 2-fold [14,15]. A high kisspeptin level was observed in PCOS females due to an over-active Kiss1 gene expression system [15].

Nuclear receptors, DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia congenital critical region on the X chromosome, gene 1) and steroidogenic factor 1 (SF1), are critical for female fetus development. DAX1 is essential for the hypothalamus-pituitary-gonadal axis, whose overexpression results in ovary development, while down expression leads to the development of the Wolffian duct, which participates in the formation of the male reproductive organ [16,17]. DAX1 mutation results in developmental abnormality, including deficient hypothalamic GnRH secretion and adrenal hypoplasia [18]. The promoter region of the DAX1 gene has two SF1 binding sites, which results in transcriptional activation [17]. SF1 protein also regulates the transcription of many other genes involved in the adrenal gland and gonad development.

An alteration in the level of steroid hormone (androgen, estrogen, progesterone, and testosterone) reportedly affects GnRH secretion and later on the development of ovarian follicles [14,15]. Nuclear receptors such as DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia congenital critical region on the X chromosome, gene 1) and SF-1also affect the level of various sex hormones and ovary formation [16,17]. Hence, in the present work, an in silico analysis was carried out to evaluate the effect of hyperandrogenicity during fetal ovarian development. The study involved evaluating the effect of these steroid hormones on the promoter region of KISS1 gene; DAX1 gene and also on the binding of kisspeptin (involved in GnRH secretion) and SF1 protein (involved in DAX1 expression modulation) using molecular docking.

2. MATERIALS AND METHODS

2.1. Molecular Modeling

There was no reported tertiary structure for kisspeptin protein (Uniprot ID: Q15726) and SF1 protein (Uniprot ID: Q13285) in the RCSB-PDB database and hence was modeled computationally. The tertiary structure of SF1 protein (Uniprot ID: Q13285) was modeled using the homology modeling tool Swiss-Model web server. No close structure with >30% sequence similarity was available for kisspeptin protein; hence, it was modeled using an Ab-initio-based modeling tool I-TASSER [19-21]. Modeled protein structures were further validated for overall structure quality using various online tools. Ramachandran plot was measured to analyze the stereochemical and overall structure quality [22]. The Q-mean score was calculated using the Swiss-Model web server for local and global analysis of modeled protein structures [23]. ProSA Z-score highlighted the overall model quality score and was calculated using ProSA server [24]. Verify 3D score was calculated for compatibility of 3D atomic model with its amino acid sequence [21]. The structures were subjected to energy minimization, to remove unfavorable non-bonded contacts, using the YASARA Energy Minimization server [25].

The promoter region of gene Kiss1 (Gene ID: 3814) and DAX1 (Gene ID: 190) was predicted using online promoter prediction servers “Neural Network promoter prediction server” [26]; “Soft berry FPROM Human promoter prediction server” [27] and ‘Promoter 2.0 prediction server’ [28]. Consensus promoter regions obtained from these servers were chosen, and tertiary structures of these promoters were modeled using the “mol2it” server and energy minimization was done using the AMBER force field [29].

The 3D structures of all studied steroids, that is, androgen, estrogen, progesterone, and testosterone with PubChem CIDs 6128, 5757, 5994, and 6013, respectively, were downloaded from the NCBI PubChem database [Figure 1]. Molecular file format converter Open Babel [30] was used to convert mol2 files of steroids to PDB files. All the structure files were subjected to “dock prep” module of UCSF Chimera v1.15 [31] for docking studies.

2.2. Molecular Docking

All selected steroids (androgen, estrogen, progesterone, and testosterone) were individually docked against modeled kisspeptin protein, SF1 protein, and kiss1 gene promoter region using Autodock v4.2.6 [32]. PDB structure of kisspeptin protein and kiss1 gene promoter regions was converted to PDBQT using MGL Autodesk tool v1.5.6 [32,33]. Only polar hydrogens were added, and charges (Kollman and Gasteiger) were assigned to maintain homogeneity throughout the structure. After assigning torsions and rotatable bonds, individual steroid structures were converted in PDBQT format. A grid box was generated with a default spacing value of 0.375Å. Using the genetic algorithm (GA) as a search parameter, a total of 100 independent runs with a step size of 0.2Å for translation and 5° for orientations and torsions were performed. The maximum number of gestations was set to 1000. The maximum number of top individuals that automatically survived was set to 1 with a mutation rate of 0.02, crossover rate of 0.8, cluster tolerance of 0.5Å, and external grid energy 1000.

The DNA promoter region of gene DAX1 was docked against free SF1 protein and SF1 complexed with different steroids (androgen, estrogen, progesterone, and testosterone) using a protein nucleotide dock module of Hex 8.0 [34-36]. Free SF1 protein and SF1 protein with steroid dock

Figure 1: Structure of studied steroid hormones – (a) androgen, (b) estrogen, (c) progesterone, and (d) testosterone.
complexes were treated as the receptor, and the DAX1 gene promoter region was uploaded as the ligand. Using shape+electro as correlation type, 0.6 as grid dimension, 180 as receptor, and ligand range with a step size of 7.5, a total of 25 searches were performed. The docking visualization and analysis were carried out using LigPlot+ [37].

3. RESULTS AND DISCUSSION

3.1. Molecular Modeling

The 3D structure of human kisspeptin protein (Uniprot ID: Q15726) was modeled by ab-initio modeling approach using the web server I-TASSER. The modeled tertiary structure of kisspeptin [Figure 2a] was selected after structure evaluation and validation [Table 1]. Ramachandran plot [Figure 3a] showed 93.4% residue in the favored and allowed region, signifying a good model, as described in earlier report [22]. Furthermore, the ProSA Z-score for the model was observed optimal of −4.46 [Figure 4a] with a satisfactory Q-mean score of −3.22. The Verify 3D pass statement confirmed no error with experimental and theoretical models of proteins, which suggested the conformational stability of protein model, in accordance with the previous findings [21,23]. The selected structure was energy minimized using the YASARA energy minimization server to remove unfavorable non-bonded contacts in concurrence to previous finding [25], and the minimized structure was used for further study.

The homology model of SF1 protein was generated using the “Swiss-Model” web server and “human nuclear receptor sf-1 (PDB: 4QJR1A)” as template, the later showing 99.18% sequence similarity with human SF1 protein (Uniprot ID: Q13285). The 3D structure of SF1 [Figure 2b] was selected after structure evaluation and validation [Table 1]. Ramachandran plot for SF1 showed 95.1% residue in the

![Kisspeptin protein](image1.png)  ![SF1 protein](image2.png)

**Figure 2:** The modeled tertiary structure of proteins – (a) kisspeptin, and (b) SF1

<table>
<thead>
<tr>
<th>Validation tools</th>
<th>Kisspeptin protein model</th>
<th>SF1 protein model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramachandran plot</td>
<td>Favored region 64.5%</td>
<td>95.1%</td>
</tr>
<tr>
<td></td>
<td>Allowed region 28.9%</td>
<td>4.5%</td>
</tr>
<tr>
<td></td>
<td>Disallowed region 6.5%</td>
<td>0.4%</td>
</tr>
<tr>
<td></td>
<td>Ramachandran Z-score −4.829</td>
<td>1.331</td>
</tr>
<tr>
<td></td>
<td>Q-mean score −3.22</td>
<td>−0.96</td>
</tr>
<tr>
<td>Verify 3D</td>
<td>Pass (89.86%)</td>
<td>Pass (84.02%)</td>
</tr>
<tr>
<td>ProSA Z-score</td>
<td>−4.46</td>
<td>−6.69</td>
</tr>
</tbody>
</table>

![Ramachandran plot](image3.png)

**Figure 3:** Ramachandran plot for modeled protein structures of (a) kisspeptin and (b) SF1.
favored region [Figure 3b] suggesting it as a good working model, in concurrence to earlier report [22]. The ProSA Z-score [Figure 4b] of −6.69 confirmed no error with the experimental and theoretical model of proteins [24]. The Q-mean score of −0.96 showed good structure prediction compared to its template. The selected structure was energy minimized using the YASARA energy minimization server to remove unfavorable non-bonded contacts in concurrence to previous finding [25] and was used for further study.

The DNA promoter regions for gene Kiss1 and DAX1 were identified using machine learning approaches with a promoter range of 3175–3225 and 1514–1564, respectively [Table 2]. The tertiary structures for both the gene promoter regions were predicted using the “modelit” web server with a straight B-DNA parameter setup and were used for further molecular docking studies [Figure 5].

3.2. Molecular Docking

All the steroids (androgen, estrogen, progesterone, and testosterone), taken for the present study, were docked against the promoter region of the kiss1 gene, kisspeptin protein, and SF1 protein. The binding affinities of free SF1 protein and its dock complex with (androgen, estrogen, progesterone, and testosterone) were estimated by docking interaction on promoter region of DAX1 gene. All studied proteins and promoter regions showed good binding of steroids with binding energies ranging from −7.50 to −9.70 Kcal/Mol.

The binding score for docking interaction of steroids (androgen, estrogen, progesterone, and testosterone) with the promoter region of Kiss1 gene ranged from −8.25 to −9.66 Kcal/Mol, where androgen showed minimum binding energy of −9.66 Kcal/Mol [Table 3]. Steroids androgen and progesterone had the same binding score and a common binding location toward the 5’ region over the TATA box (A [a13, t14, a15, t16], B [a37, t38, a39]). In contrary, the steroids – estrogen and testosterone, showed similar binding score and had the same binding site with common interacting residues toward the center of the promoter region (A [t23, c24, t25], B [g27, a28, t29, g30]) [Figure 6]. The androgen and progesterone binding over TATA box may be predicted to prevent binding of TATA box binding protein which may inhibit the expression of kiss1 gene. In contrast, testosterone and estrogen bind downstream of the TATA box and do not appear

Figure 4: ProSA model quality score graph for modeled protein structures – (a) kisspeptin (Z-score= −4.46) and (b) SF1 (Z-score= −6.69).

Figure 5: The modeled tertiary structure of promoter regions of gene (a) Kiss1 and (b) DAX1.
Table 2: Promoter prediction score for genes – Kiss1 and DAX1.

<table>
<thead>
<tr>
<th>Promoter prediction servers</th>
<th>Kiss1 gene</th>
<th>DAX1 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>3175</td>
<td>1514</td>
</tr>
<tr>
<td>End</td>
<td>3225</td>
<td>1564</td>
</tr>
<tr>
<td>Promoter position</td>
<td>3215</td>
<td>1549</td>
</tr>
<tr>
<td>Neural network promoter prediction score</td>
<td>0.90</td>
<td>0.99</td>
</tr>
<tr>
<td>FPROM human promoter prediction score</td>
<td>7.798</td>
<td>8.921</td>
</tr>
<tr>
<td>Promoter 2.0 prediction score</td>
<td>1.166</td>
<td>1.244</td>
</tr>
<tr>
<td>LDF</td>
<td>+3.608</td>
<td>+7.283</td>
</tr>
</tbody>
</table>

Table 3: Binding energy and interacting residues of Kiss1 gene promoter region when docked against studied steroid hormones (androgen, estrogen, progesterone, and testosterone).

<table>
<thead>
<tr>
<th>Steroids</th>
<th>Binding energy (KCal/Mol)</th>
<th>H-bond forming residues</th>
<th>Interacting residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen</td>
<td>−9.66</td>
<td>-</td>
<td>A (a13, t14, a15, t16), B (a37, t38, a39)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>−8.25</td>
<td>B (g27 (3.01Å))</td>
<td>A (t23, c24, t25), B (g27, a28, t29, g30)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>−9.62</td>
<td>-</td>
<td>A (a13, t14, a15, t16), B (a37, t38, g39, g40, g41)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>−8.27</td>
<td>-</td>
<td>A (t23, c24, t25), B (g27, a28, t29, g30, c31)</td>
</tr>
</tbody>
</table>

Table 4: Binding energy and interacting residues of kisspeptin protein when docked against studied steroid hormones (androgen, estrogen, progesterone, and testosterone).

<table>
<thead>
<tr>
<th>Steroids</th>
<th>Binding energy (KCal/Mol)</th>
<th>H-bond forming residues</th>
<th>Interacting residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen</td>
<td>−8.80</td>
<td>Phe117 (2.91Å), Leu119 (2.72Å)</td>
<td>Leu44, Ala45, Pro46, Gly47, Glu48, Leu51, Cys53, Glu55, Thr61, Phe117, Gly118, and Leu119.</td>
</tr>
<tr>
<td>Progesterone</td>
<td>−9.30</td>
<td>-</td>
<td>Gln8, Leu9, Leu11, Phe12, Pro84, Gly85, Leu86, Ser87, Ala100, Val101, Leu102, Phe121, and Ala127.</td>
</tr>
<tr>
<td>Testosterone</td>
<td>−9.38</td>
<td>Phe117 (2.88Å), Leu119 (2.74Å)</td>
<td>Leu44, Ala45, Pro46, Glu48, Leu51, Cys53, Glu55, Thr61, Phe117, Gly118, and Leu119.</td>
</tr>
</tbody>
</table>

Figure 6: Docking interactions of the KISS1 gene promoter region with steroid hormones – (a) androgen, (b) estrogen, (c) progesterone, and (d) testosterone.

Figure 7: Docking interactions of the KISS1 gene promoter region with steroid hormones – (a) androgen, (b) estrogen, (c) progesterone, and (d) testosterone.

signaling in the hypothalamus and downstream regulation of the hypothalamic-pituitary-gonads axis to cause an imbalanced steroid hormone level.

The binding score of steroids (androgen, estrogen, progesterone, and testosterone) when docked against kisspeptin protein ranged from −8.80 to −9.46 Kcal/Mol. Androgen showed minimum binding energy of −9.46 Kcal/Mol, followed by testosterone, progesterone, and estrogen with a binding energy of −9.38, −9.30, and −8.80 Kcal/Mol, respectively [Table 4]. Androgen and progesterone showed the same binding pockets represented by 9-amino acids: Gln8, Leu9, Leu11, Phe12, Pro84, Leu86, Ser87, Val101, and Leu102. Estrogen and testosterone showed another common binding pocket represented by Leu44, Ala45, Pro46, Glu48, Leu51, Cys53, Glu55, Thr61, and Gly118 as common nearby residue including Phe117 and Leu119 as H-bond forming amino acids [Figure 8]. Kisspeptin to regulate Kiss1 gene expression [Figure 7]. This androgen- and progesterone-mediated Kiss1 gene regulation may affect kisspeptin
protein region 112–121 (kisspeptin-10) has been observed earlier as critical for binding to its receptor (Gpr54) [38-40]. Our studied hormones (estrogen, progesterone, and testosterone) showed their binding in and around the receptor-binding region of kisspeptin protein, which may be predicted to hamper kisspeptin binding to its natural receptor. This can be due to the negative feedback mechanism of sex steroids to regulate their secretion by inhibiting kisspeptin binding to its receptor [41]. However, androgen was found to bind at different location, leaving receptor binding pocket free to trigger kisspeptin binding to its receptor. Thus, it may be inferred that in the case of hyperandrogenism, androgen outnumbered other sex steroids to regulate GnRH surge. This altered GnRH surge may further produce imbalanced LH and FSH hormones through the overstimulated hypothalamus-pituitary-gonadal axis.

The docking interactions of steroids (androgen, estrogen, progesterone, and testosterone) against SF1 protein had the binding scores in the range of −7.59−9.51 Kcal/Mol. Progesterone showed minimum binding energy of −9.51 Kcal/Mol followed by androgen, testosterone, and estrogen with a binding energy of −8.78, −8.38, and −7.59 Kcal/Mol, respectively [Table 5]. Androgen, progesterone, and testosterone showed the same binding pocket with Leu306 and His310 as nearby interaction residues of SF1 protein when docked against studied steroid hormones (androgen, estrogen, progesterone, and testosterone).

Table 5: Binding energy and interacting residues of SF1 protein when docked against studied steroid hormones (androgen, estrogen, progesterone, and testosterone).

<table>
<thead>
<tr>
<th>Steroids</th>
<th>Binding energy (KCal/Mol)</th>
<th>H-bond forming residues</th>
<th>Interacting residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen</td>
<td>−7.59</td>
<td>Met446 (2.70Å), Glu454 (3.08Å)</td>
<td>His441, Asn444, Met446, Pro447, Arg448, Asn449, Asn450, Ile453, and Glu454.</td>
</tr>
<tr>
<td>Testosterone</td>
<td>−8.38</td>
<td>Leu306 (2.80Å), Asp309 (2.98Å), Tyr436 (3.01Å)</td>
<td>Leu265, Met268, Ala269, Thr272, Leu306, Asp309, His310, Val326, and Tyr436.</td>
</tr>
</tbody>
</table>

Figure 7: Steroid hormones binding over promoter region of KISS1 gene.

Figure 8: Dock interactions of kisspeptin protein with steroid hormones – (a) androgen, (b) estrogen, (c) progesterone, and (d) testosterone.

Figure 9: Dock interactions of SF1 protein with steroid hormones – (a) androgen, (b) estrogen, (c) progesterone, and (d) testosterone.
amino acid residues, while estrogen showed separate binding pocket [Figure 9]. All studied sex steroids showed their binding in the leucine-rich ligand-binding domain of SF1 protein, which might disturb SF1 binding to the promoter region of the DAX1 gene, as reported earlier [42,43]. The high binding affinity of progesterone, androgen, and testosterone toward SF1 protein binding can be predicted to be crucial for expressing other gonadal developmental genes.

Docking analysis of SF1 protein and SF1 protein complexed with individual steroid (androgen, estrogen, progesterone, and testosterone) against the promoter region of DAX1 gene was done using Hex 8.0. The unbound SF1 protein showed a comparative minimum binding score of −1299.84 against the DAX1 gene promoter region compared to SF1 protein bound with steroid [Table 6]. SF1 protein showed two different binding positions when docked against the DAX1 gene promoter region. The unbound SF1 protein and SF1 protein bound to progesterone showed binding at 5' upstream region near TATA box whereas the other three studied hormones bound to SF1 protein showed their binding interactions at 3' region of promoter region, near the transcription start site [Figure 10a-e]. DAX1 gene promoter region has 2 SF1 binding sites for the expression of the DAX1 gene [17]. Dax1 is crucial for expressing oogenesis regulated genes, steroidal differentiation-related genes, and sex differentiation [44,45]. The unbound SF1 protein showed maximum binding affinity near the TATA box of DAX1 gene, reflecting possible overexpression of the DAX1 gene in its presence, further stimulating other genes (StAR, Cyp1, and Cyp19) expression and development of the normal ovary. Earlier studies have showed that DAX1 mutant mice show a high expression of steroidogenic genes, including StAR, P450c17, P450scc, and 3β-HSD [18,46], which trigger testosterone synthesis and disturb normal oogenesis in females. The SF1 protein when complexed with studied steroids showed significantly less binding affinity [Table 6], thus hinting toward possible downregulation of DAX1 gene expression.

4. CONCLUSION

PCOS is a multifactorial disorder, but hyperandrogenism is the significant cause. The observations of present in silico investigation reflect that the binding of steroid hormones with SF1 protein lowers the expression of DAX1 gene. The SF1 protein is essentially required for DAX1 gene expression, and the binding of studied steroid hormones with SF1 protein may result in downregulation of DAX1 gene expression, leading to abnormal ovary development in a female fetus as well as abnormal sex steroids level. Thus, it may be concluded that over-secretion of steroid hormones (androgen, estrogen, progesterone, and testosterone) possibly affects female
Table 6: Binding energy and interacting residues of the promoter region of DAX1 gene when docked against unbound SF1 protein and SF1 protein bound to studied steroid hormones (androgen, estrogen, progesterone, and testosterone).

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Binding score</th>
<th>H-bond forming residues</th>
<th>Interacting residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>−1299.84</td>
<td></td>
<td>A (c2, t3, g4, c5, g6, t7, g8, c9, g10, c11, g12).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B (a37, g38, c39, g40, c41, g42, c43, a44, c45, g46, c47, a48).</td>
</tr>
</tbody>
</table>

(Continued)
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12. REFERENCES

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