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Identification of Potential Cytochrome p450c 17 Alpha Inhibitors for the Treatment of PCOS via Scaffold Hopping and Fragment-Based De-Novo Drug Design

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Abstract: Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting reproductive-aged women. It is characterized by hyperandrogenism, ovarian dysfunction, and metabolic abnormalities. Current treatment options have limitations and many women remain undiagnosed or untreated. Cytochrome P450c 17 alpha (CYP17A1) plays a key role in androgen biosynthesis and is a potential therapeutic target for PCOS. Known CYP17A1 inhibitors metformin, spironolactone, and clomiphene were used for scaffold hopping to generate structurally diverse compounds. These were screened against CYP17A1 (PDB code 3RUK) through molecular docking. Hits were subjected to fragment-based de novo design and further docking. Quality parameters, ADMET profiling, and biological activity predictions were evaluated. Scaffold hopping yielded 300 compounds, from which 10 hits were identified. De novo design generated 326 ligands, of which 7 demonstrated superior binding to 3RUK compared to reference drugs. These hits formed favourable interactions within the binding pocket and exhibited drug-like properties. They were predicted to inhibit CYP17A1 and show activity for PCOS-related indications. Toxicity profiling suggested an acceptable safety profile. Through an integrated in silico workflow, this study identified 7 novel CYP17A1 inhibitor scaffolds as potential leads for PCOS treatment. Their predicted bioactivities and properties warrant further experimental validation. This approach provides a foundation for the development of improved PCOS therapeutics targeting androgen biosynthesis.

Keywords: 17-Alpha Hydroxylase, P-Glycoprotein, Chemotypes and Hepatotoxicity

1. Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder among premenopausal women in women where their balance of androgens is disrupted, leading to excessive production or impaired metabolism of these hormones [47]. The infection is analysed using the National Institutes of Health criteria, Rotterdam standards, and Androgen Excess Society criteria. Be that as it may, these rules can't be applied to teenagers because of physiological changes related to adolescence covering PCOS obsessive changes [31]. Although the exact cause of PCOS is still mostly unknown, a growing body of research indicates that it may be a complex multigenic disorder with significant environmental and epigenetic influences, including lifestyle and nutrition choices. Abdominal adiposity, infertility, abnormal bleeding, increased pregnancy loss, and complications of pregnancy insulin resistance, obesity, metabolic diseases, and cardiovascular risk factors are commonly characterized by PCOS.

PCOS is a multifaceted condition that requires a comprehensive diagnostic approach considering ultrasound findings, hyperandrogenism markers, anovulation patterns, and insulin resistance [26]. Understanding the associated complications, including insulin resistance, abnormal lipid profiles, and android-type obesity, is crucial for effective long-term management and prevention of related health issues [26]. A clinical feature of polycystic ovarian syndrome (PCOS) is androgen excess, and CYP17A1 is closely linked to and significantly communicates with male pattern hair loss. The excessive manifestation of PI3K/AKT symptoms may lead to ovarian dysfunction [49]. An essential enzyme in humans that generates endogenous androgens is cytochrome P450 17A1. In the course of enzymatic catalysis, the enzyme initially engages with pregnenolone and progesterone substrates, specifically affecting hydroxylation at the C17 position. This results in the generation of 17-hydroxypregnenolone and 17-hydroxyprogesterone. Subsequently, the enzyme facilitates the cleavage of the C17-C20 bond within 17-hydroxypregnenolone and 17-hydroxyprogesterone, leading to the formation of dehydroepiandrosterone and androstenedione. Currently, some CYP17A1 inhibitors available are abiraterone acetate and spironolactone. These inhibitors have a steroidal scaffold that is similar to endogenous substrates, however, they also have a lot of adverse effects. These include diarrhoea, low potassium, high blood pressure, vomiting, edema, and increased hyperglycemia and joint discomfort. Abiraterone also demonstrates promiscuity by blocking several different CYP enzymes, which may result in side effects such as arrhythmias, liver failure, adrenal insufficiency, and heart problems. Abiraterone's current drawbacks highlight the necessity of continued research into creating CYP17A1 inhibitors of the next generation with improved selectivity and fewer side effects [49].

This condition is treated with a variety of synthetic medications, including metformin and clomiphene; however, these medications have adverse consequences, including congenital heart disease. Therefore, infertility is treated with natural chemicals that have no negative side effects [22].

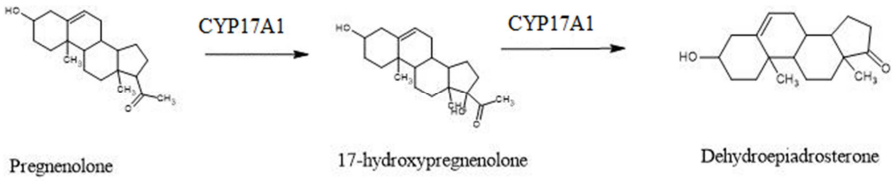


Figure 1. *Conversion of pregnenolone to dehydroepiandrosterone.*

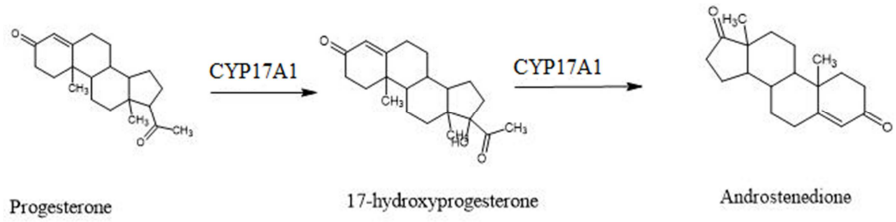


Figure 2. *Conversion of progesterone to dehydroepiandrosterone.*

2. Materials and Methods

2.1. Materials

The strategy employed for identifying potential inhibitors against Cytochrome P450c 17 alpha is illustrated in Figure 3. Metformin, spironolactone, and clomiphene, known inhibitors of Cytochrome P450c 17 alpha, were utilised for scaffold hopping through the Balanced Rapid and Unrestricted Server for Extensive Ligand-Aimed Screening (BRUSELAS) [3]. BRUSELAS, a web architecture, integrates the Weighted Gaussian Algorithm (WEGA), ligand similarity using clique algorithm (LiSiCA), Screen3D, and OptiPharm algorithms to conduct shape similarity searching and pharmacophore screening. Subsequently, a structural similarity search was performed via DrugBank [48, 23] and the Zinc15 database [38] for the identified nonsteroidal ligands from the scaffold hopping to obtain diverse structures for each core structure. These chemical entities were then docked onto the prepared and energy-minimized P450c 17 alpha (code 3RUK) obtained from the Research Collaboratory for Structural Bioinformatics Protein Database (RCSB PDB) [6]. Compounds demonstrating low binding energy and proper orientation in the binding site were selected for de novo design and further docking onto the protein. The novel hit compounds chosen will undergo evaluation for ADMET profiles, biological activity predictions, and quality parameter computations.

2.2. Receptor Retrieval and Protein Preparation

The X-ray crystal structure (PDB code: 3RUK, resolution 2.80A˚) of Cytochrome P450c 17 alpha bound to abiraterone abduct was retrieved from RCSB PDB [35]. Protein preparation involved deleting ligand and water molecules using BIOVIA Discovery Studio Visualizer v19.1.0.18287 [39], visualising the energy-minimized structure, removing water molecules solvating the protein, and saving it in Protein Data Bank (PDB) format, which was later converted to AutoDock Vina's [40] compatible PDBQT format for

molecular docking.

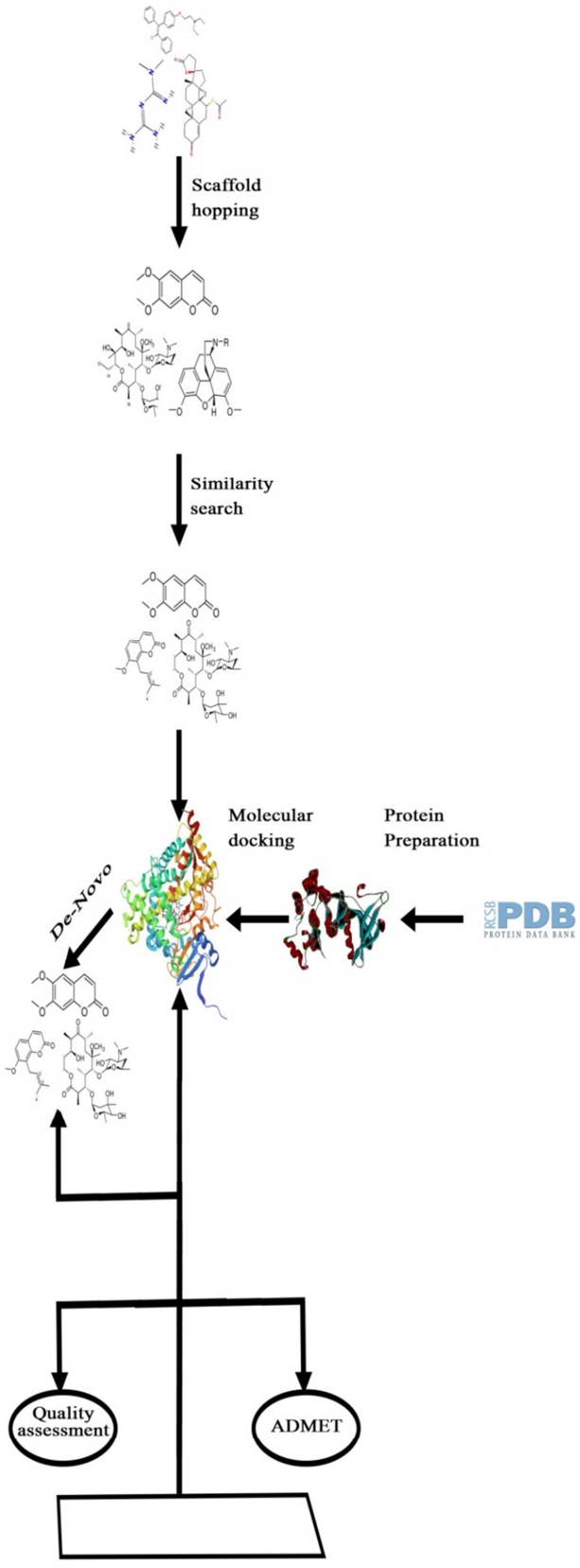


Figure 3. *Detailed methodology scheme including scaffold hopping, fragment-based de novo design, and molecular docking in the identification of potential inhibitors of 3RUK.*

2.3. Scaffold Hopping

To discover new chemotypes without the steroidal core, the sdf format of approved drugs—metformin, spironolactone, and clomiphene—was submitted to BRUSELAS [3]. Among the 300 generated scaffolds, 117 nonsteroidal scaffolds and their structural derivatives from ChemSpider, [29] Pub Chem [23], DrugBank [48], and Zinc15 databases [38] were selected for affinity prediction using AutoDock Vina [40].

2.4. Molecular Docking Studies

The molecular docking studies involved two phases, with the first phase comprising 2276 compounds, including known drugs and compounds from BRUSELAS and their derivatives. The second phase involved compounds from de novo design. Ligands and proteins were prepared using AutoDock Tools [16], and molecular docking was conducted with AutoDock Vina [40].

2.5. Fragment-Based De Novo Design

Protein–ligand complexes from scaffold hopping were input into eLEA3D [13] for fragment-based de novo design. The binding site radius was set to 15A˚, and molecular interactions between hits and 3RUK were elucidated using BIOVIA Discovery Studio Visualizer v19.1.0.18287 [39].

2.6. Quality Assessment

Ligand efficiency metrics, including inhibitory constant, ligand efficiency, ligand efficiency scale, and ligand efficiency-dependent lipophilicity, were computed following established protocols [34].

2.7. ADMET Determination

Toxicity properties were determined using ProTox II [4] and OSIRIS Property Explorer in Data Warrior [35], while ADME properties were estimated using SwissADME [10].

2.8. Biological Activity Predictions and Exploration of Potential Hit Compounds

The biological activities of hits were predicted using the simplified molecular input line entry system (SMILES) with the Prediction of Activity Spectra for Substance (PASS) [24]. DrugBank [48] was screened for structural derivatives to identify drugs explored for PCOS treatment and potential mechanisms of action from similar compounds.

3. Results and Discussion

This section presents the outcomes of diverse in silico techniques, encompassing scaffold hopping, de novo design, molecular docking, ADMET profiling, biological activity predictions, and quality assessment of the identified hit compounds.

3.1. Scaffold Hopping

The application of scaffold hopping to metformin, spironolactone, and clomiphene using BRUSELAS [3] resulted in 300 compounds from the ChEMBL database [11] with varying similarity scores. Virtual screening of the 2276 nonsteroidal compounds against the target protein led to the identification of 10 hits. These hits were selected based on their depth of docking into the receptor binding site and binding energy.

3.2. Fragment-Based De Novo Design

Fragment-based de novo design involves linking small molecules fitting various constraint portions in a protein target's binding site to form a drug lead [46]. This approach yields novel and potent inhibitors with improved activities against their target, a cost- and time-efficient manner that has shown promise in various human ailments [34, 9]. In the context of PCOS treatment, the need for novel compounds with alternative core structures, prompted by the potential cytotoxicity of the steroidal core of spironolactone on the cholesterol biosynthetic pathway, underscores the significance of de novo design [34].

In this research, 27 compounds obtained from scaffold hopping with lower or comparable binding energy to spironolactone, metformin, and clomiphene were subjected to e-LEA3D [13] for de novo design. A total of 326 potential novel compounds were generated, filtered based on Lipinski's rule of five (Ro5), and reduced to 124 compounds for subsequent molecular docking studies. Among these, 7 compounds (Table 2) demonstrated deep docking into the binding pocket of the 3RUK protein and exhibited binding energy lower than spironolactone (−9.0 kcal/mol), clomiphene (−6.7 kcal/mol), and metformin (−4.7 kcal/mol). Notably, the binding energy of the hit compounds, ranging from −9.9 kcal/mol to −10.1 kcal/mol, suggests their potential to inhibit 3RUK for PCOS treatment. AutoDock Vina docking studies indicate that ligands with binding energy ≤−7.0 kcal/mol to the protein of interest have demonstrated significant biological activity against the target [8]. Additionally, a search through public databases, including ChemSpider [28], Zinc15 [38], PubChem [23], or DrugBank [48], revealed no duplicates of these compounds, highlighting the uniqueness of the selected hit compounds.

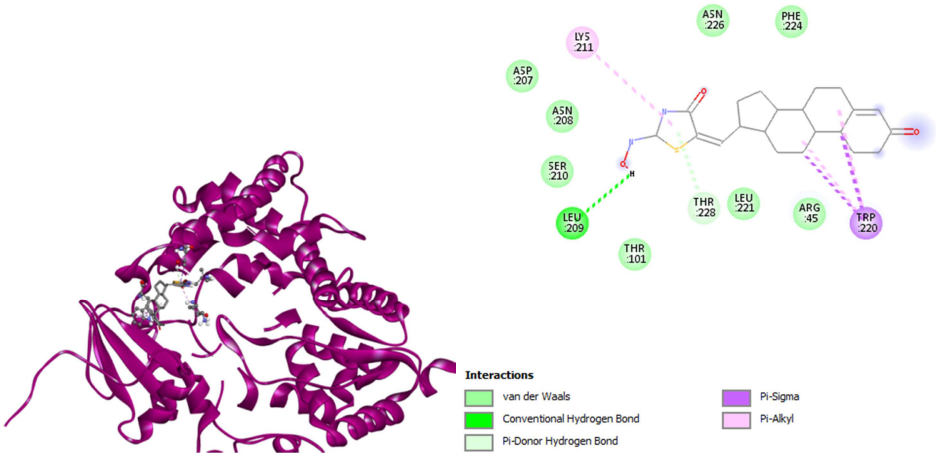


Figure 4. *The cartoon representations and 2D interactions of the 3RUK-hits complexes as visualized in Discovery Studio v19.1.0.18287: Cartoon of 3RUK-B3 complex, 2D interaction of 5αR-2-A1 complex, and legend of interactions.*

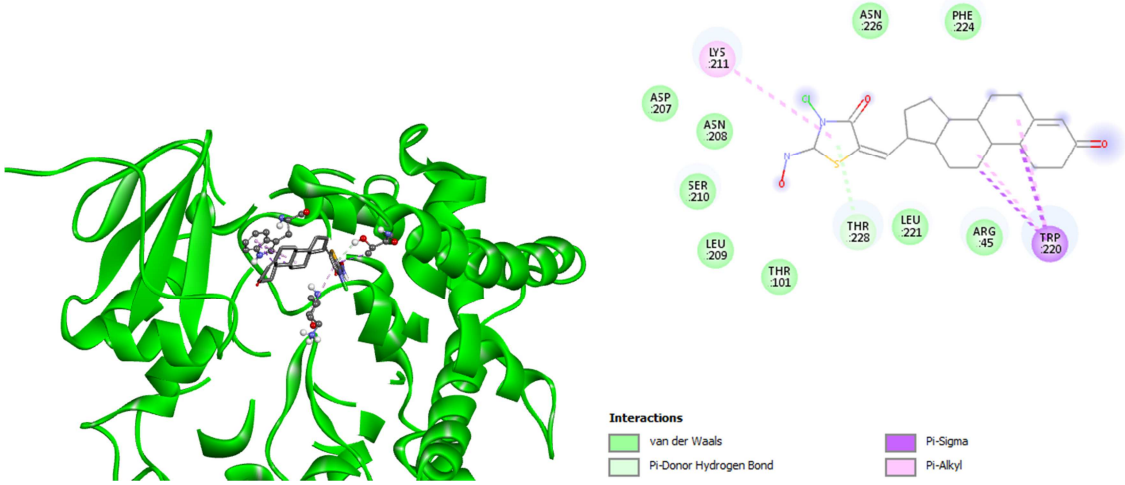


Figure 5. *The cartoon representations and 2D interactions of the 3RUK-hits complexes as visualized in Discovery Studio v19.1.0.18287: Cartoon of 3RUK-B1 complex, 2D interaction of 3RUK-B1 complex, and legend of interactions.*

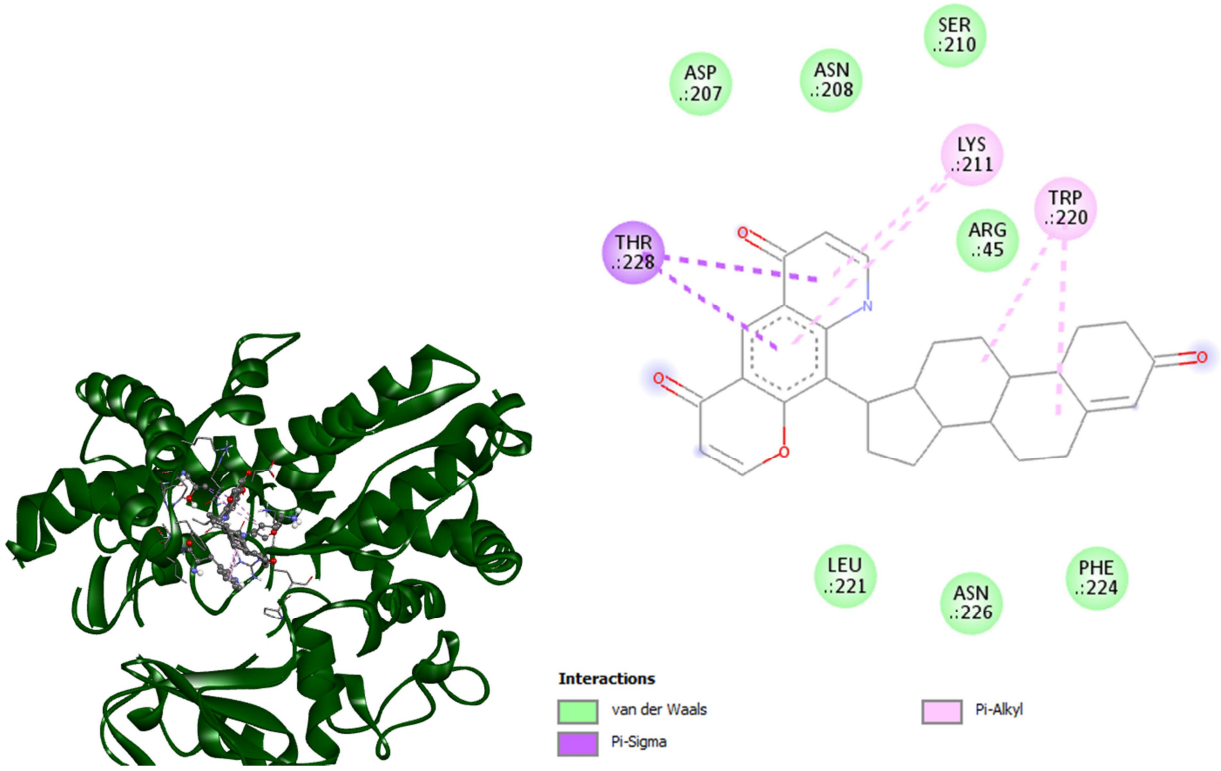


Figure 6. *The cartoon representations and 2D interactions of the 3RUK-hits complexes as visualized in Discovery Studio v19.1.0.18287: Cartoon of 3RUK-B2 complex, 2D interaction of 3RUK-B2 complex, and legend of interactions.*

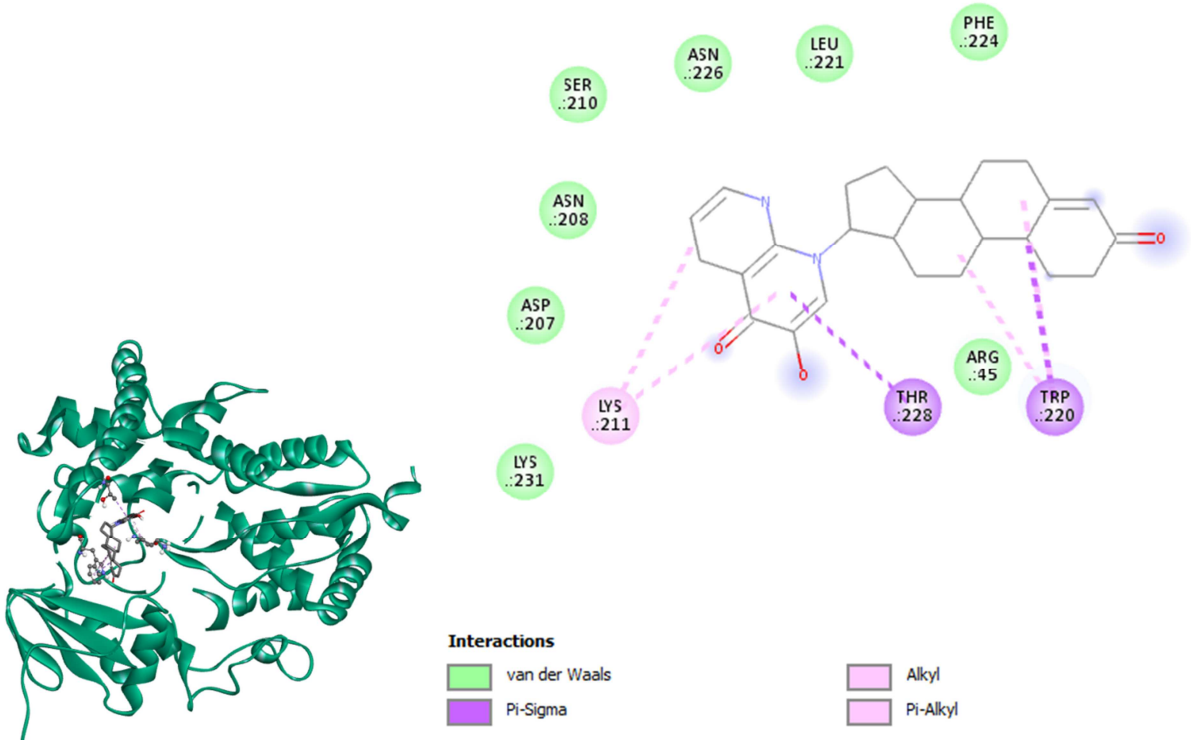


Figure 7. *The cartoon representations and 2D interactions of the 3RUK-hits complexes as visualized in Discovery Studio v19.1.0.18287: Cartoon of 3RUK-B4 complex, 2D interaction of 3RUK-B4 complex, and legend of interactions.*

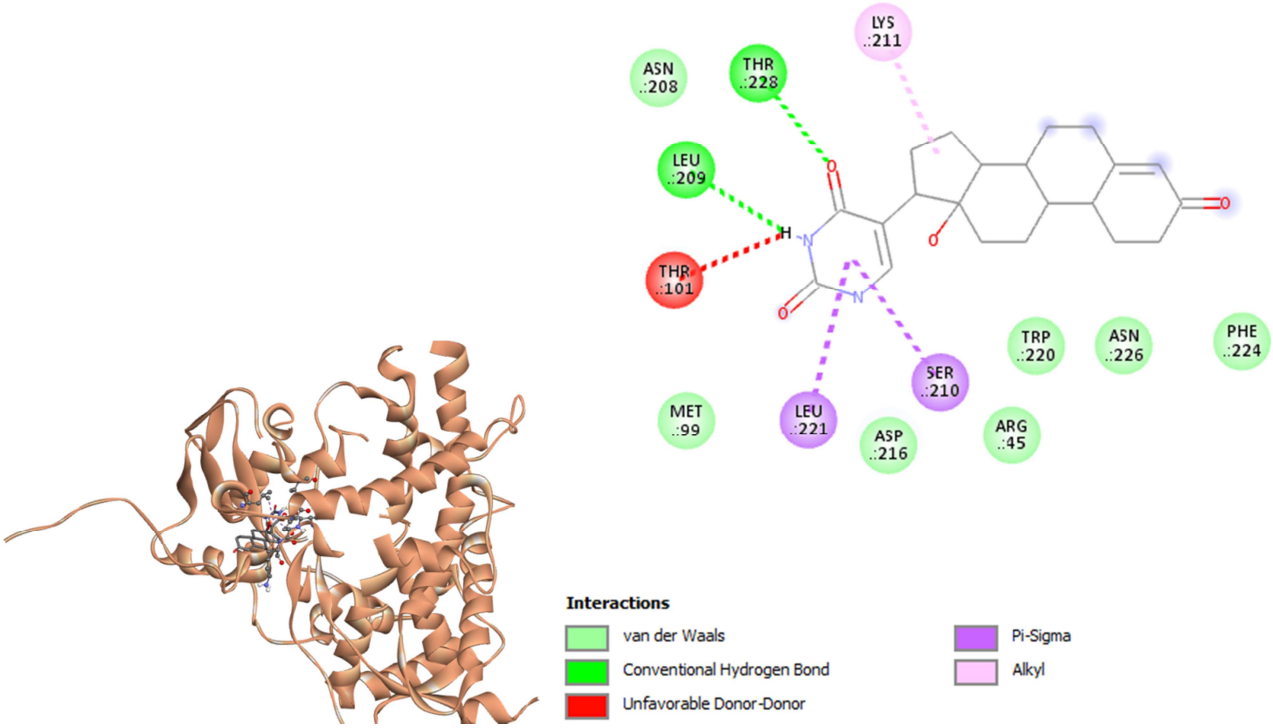


Figure 8. *The cartoon representations and 2D interactions of the 3RUK-hits complexes as visualized in Discovery Studio v19.1.0.18287: Cartoon of 3RUK-B5 complex, 2D interaction of 3RUK-B5 complex, and legend of interactions.*

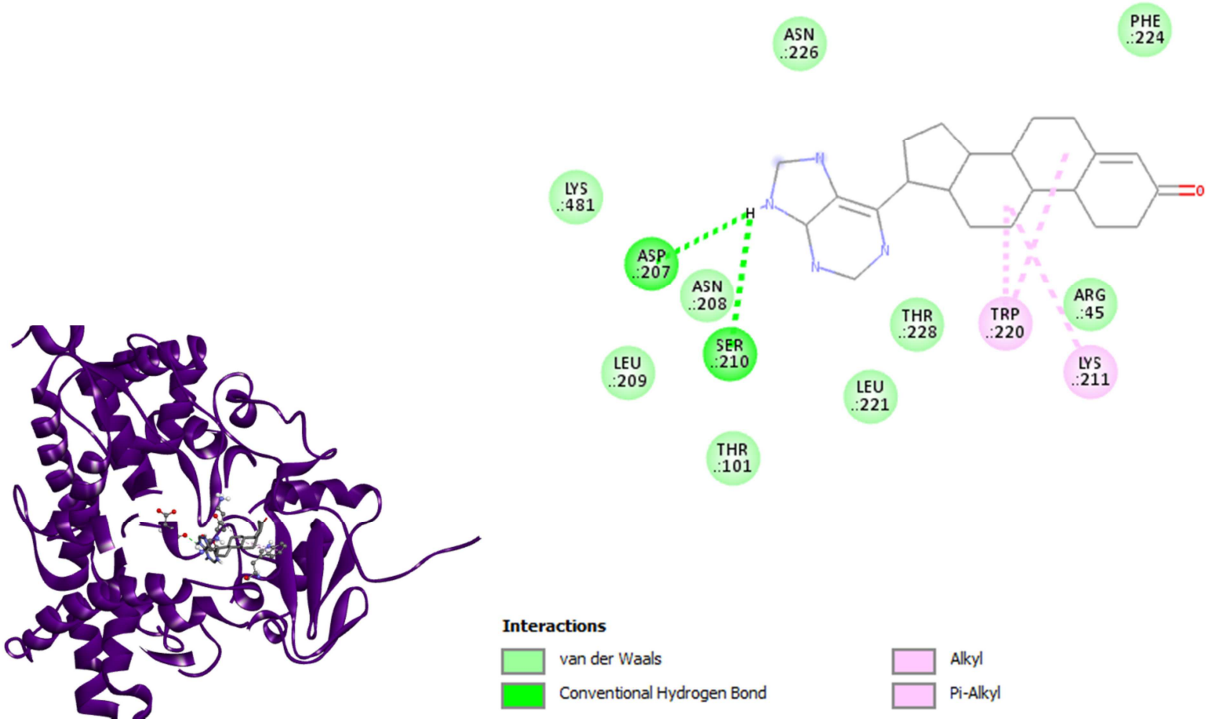


Figure 9. *The cartoon representations and 2D interactions of the 3RUK-hits complexes as visualized in Discovery Studio v19.1.0.18287: Cartoon of 3RUK-B6 complex, 2D interaction of 3RUK-B6 complex, and legend of interactions.*

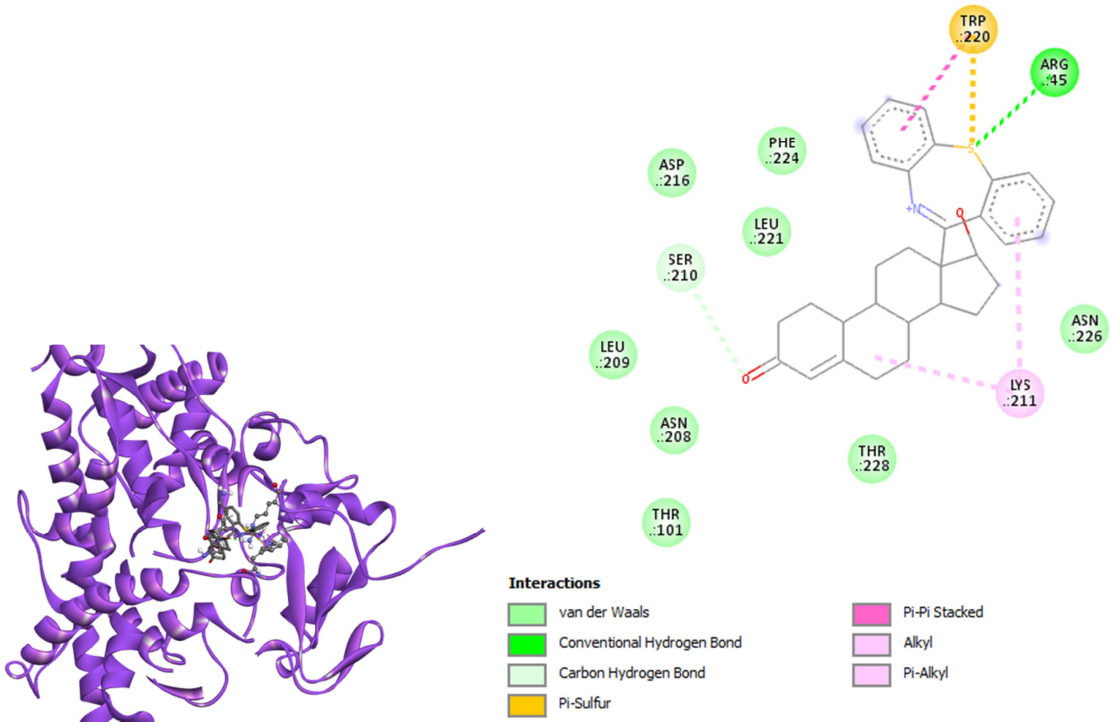


Figure 10. *The cartoon representations and 2D interactions of the 3RUK-hits complexes as visualized in Discovery Studio v19.1.0.18287: Cartoon of 3RUK-B7 complex, 2D interaction of 3RUK-B7 complex, and legend of interactions.*

3.3. Characterization of Binding Interactions

The meticulous examination of protein-ligand interactions at the atomic level represents a crucial step in designing compounds with the requisite specificity for the target of interest [14]. The stability of protein-ligand complexes hinges on the intricate interactions between the functional groups of the ligand and the amino acid residues within the protein's binding site [14]. Moreover, the presence of hydrogen bonds and hydrophobic interactions significantly contributes to the overall stabilization of the protein-ligand complex [43]. Employing the Discovery Studio software [39], we visualized two-dimensional (2D) interactions within protein-ligand complexes. This detailed analysis unveiled that the identified hit compounds engage in vital interactions with key amino acids such as Phe224, Trp220, Arg45, Leu221, and others, strategically located in the binding pocket of the 3RUK protein.

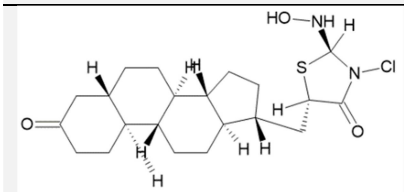
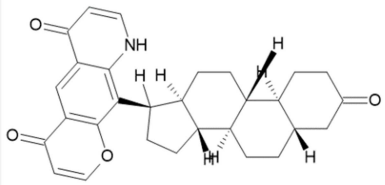
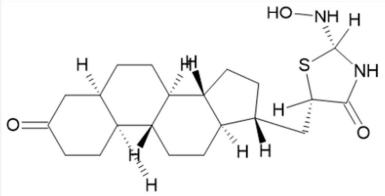
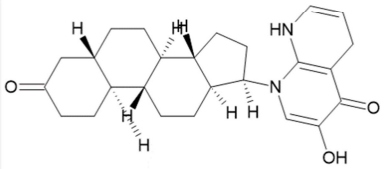
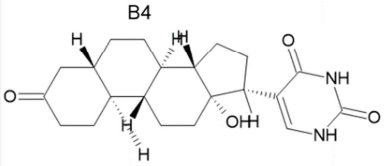
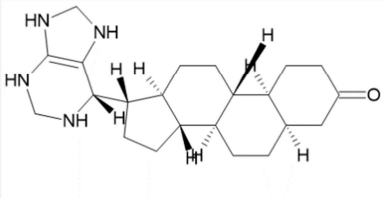
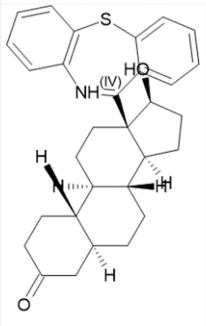
The diverse interactions observed within the protein-ligand complexes include pi-alkyl, pi-sigma, carbon-hydrogen, van der Waals, water-hydrogen, Unfavorable donor-acceptor, and hydrogen bonding. To conduct molecular docking studies, a curated selection of 2276 scaffolds derived from BRUSELAS, the Zinc database, DrugBank, and ChemSpider were utilized with AutoDock Vina. The resultant structures of the protein-ligand complexes were further scrutinized and visualized using PyMol and Discovery Studio.

Among the top seven compounds, those exhibiting lower binding energies were prioritized for in-depth investigation. For instance, Compound B3 demonstrated a binding energy of -9.6 kcal/mol, establishing hydrogen and hydrophobic bond interactions with Leu209 and Phe224, Trp220, Arg45, Leu221, Thr228, Thr101, Ser210, Asn208, Asp207, Lys211, Asn226, respectively. Similarly, B5 showcased hydrogen and hydrophobic interactions with Leu209, Thr228, and Phe224, Asn226, Trp220, Arg45, Ser210, Asp216, Leu221, Met99, Thr101, Asn208, Lys211. Compounds B5, B6, and B7 exhibited a binding energy of -9.3 kcal/mol, with B6 forming hydrophobic interactions with Phe224, Arg45, Lys211, Trp220, Thr228, Leu221, Thr101, Leu209, Asn208, Lys481, Asn226 and hydrogen bond interaction with Ser210, Asp207. B7 also displayed hydrophobic bond interactions with Asn226, Lys211, Thr228, Thr101, Asn208, Leu209, Ser210, Leu221, Asp216, Phe224, Trp220 and a hydrogen bond interaction with Arg45.

Additionally, B1 exhibited hydrophobic interactions with Phe224, Trp220, Arg45, Leu221, Thr228, Thr101, Leu209, Ser210, Asn208, Asp207, Lys211, Asn226, yielding a binding energy of -10.1 kcal/mol. B2, with a binding energy of -9.9 kcal/mol, engaged in hydrophobic interactions with Phe224, Asn226, Leu221, Thr228, Asp207, Asn208, Ser210, Lys211, Arg45, Trp220. Furthermore, B4, featuring a binding energy of -9.4 kcal/mol, formed hydrophobic interactions with Trp224, Arg45, Thr228, Lys211, Lys231, Asp207, Asn208, Ser210, Asn226, Leu221, Phe224.

In summary, the detailed analysis of protein-ligand interactions highlights the strategic binding of the identified hit compounds within the 3RUK protein's binding pocket, providing valuable insights for further exploration and development.

Table 1. *The de novo designed hit compounds and the binding energy resulting from the interactions between the amino acids in the pocket of the target protein and the ligand.*

Compound	Binding energy (kcal/mol)	Binding residues	
		Hydrogen bonding	Hydrophobic bonding
 B1	-10.1		Phe224, Trp220, Arg45, Leu221, Thr228, thr101, Leu209, Ser210, Asn208, Asp207, Lys211, Asn226
 B2	-9.9		Phe224, Asn226, Leu221, Thr228, Asp207, Asn208, Ser210, Lys211, Arg45, Trp220
 B3	-9.6	Leu209	Phe224, Trp220, Arg45, Leu221, Thr228, Thr101, Ser210, Asn208, Asp207, Lys211, Asn226
 B4	-9.4		Trp224, Arg45, Thr228, Lys211, Lys231, Asp207, Asn208, Ser210, Asn226, Leu221, Phe224
 B5	-9.3	Leu209, Thr228	Phe224, Asn226, Trp220, Arg45, Ser210, Asp216, Leu221, Met99, Thr101, Asn208, Lys211
 B6	-9.3	Ser210, Asp207	Phe224, Arg45, Lys211, Trp220, Thr228, Leu221, Thr101, Leu209, Asn208, Lys481, Asn226
 B7	-9.3	Arg45	Asn226, Lys211, Thr228, Thr101, Asn208, Leu209, Ser210, Leu221, Asp216, Phe224, Trp220

3.4. Quality Assessment

In the past, hit selection and optimization primarily relied on affinity [37]. However, this approach has limitations as affinity tends to correlate with molecular size [37]. To assess the potential of de novo designed ligands as lead molecules, various quality parameters were computed, including inhibitory constant (Ki), ligand efficiency (LE), ligand efficiency scale (LE_Scale), fit quality (FQ), and ligand efficiency-dependent lipophilicity (LELP).

The inhibitory constant (Ki), representing the concentration required for half maximum inhibition, offers insights into the likelihood of a ligand inhibiting the target [20]. Ki was calculated for all protein-ligand complexes using a modified version of the equation (1), similar to a previous study [34].

$$k_i = e^{\left(-\frac{\Delta G}{RT}\right)}$$

With a low Ki predicted for the ligands (Table 2)

Ligand efficiency (LE) expresses the binding energy normalized by the compound's size and is crucial in hit screening, as larger compounds may show greater binding energy without necessarily being the most efficient binders [19]. LE was computed using Equation (2), where BE is the binding energy and NHA is the number of heavy atoms [1].

$$LE = \frac{-BE}{\mathbb{N}HA}$$

Interestingly, all ligands fell within the optimal range (LE < 0.3 kcal/mol/HA) for the ligand efficiency of lead-like molecules [37]. However, considering that LE being size-dependent may not accurately reflect binding energy, ligand efficiency scaling (LE_Scale) was computed.

LE_Scale, a size-independent parameter, compares ligands using an exponential function to the maximal LE values [32]. LE_Scale values for all seven molecules were predicted to be in the range of 0.2 to 0.4.

$$\text{LE\_Scaling} = 0.873e^{-0.026\text{NHA}} - 0.064$$

FQ, another size-independent parameter, determines optimal ligand binding within the receptor active site [37]. All compounds were predicted to have an FQ score above 0.8, indicating stronger ligand binding. With FQ scores close to 1, optimal ligand binding is suggested.

$$FQ = \frac{LE}{LE\_SCALE}$$

LELP assesses the binding energy in relation to the compound's lipophilicity and is used to estimate drug-likeness [19]. All analogs had LELP above 3 (Table 2), suggesting optimized affinity concerning lipophilicity, by the recommended range for promising molecules [37].

$$LELP = \frac{log\ P}{LE}$$

Table 2. *Quality parameters including Ki, LE, Le_Scale, FQ, and LELP for the de novo designed hit compounds.*

Compound	B.E	Ki (nM)	LE	LE_Scale	FQ	LELP
B1	-10.1	0.025	0.361	0.356	1.014	9.524
B2	-9.9	0.018	0.291	0.297	0.980	7.624
B3	-9.6	0.011	0.356	0.369	0.965	4.656
B4	-9.4	0.008	0.313	0.336	0.932	9.436
B5	-9.3	0.007	0.344	0.369	0.932	13.032
B6	-9.3	0.007	0.344	0.369	0.932	8.730
B7	-9.3	0.007	0.274	0.297	0.923	22.540
Spironolactone	-9.0	0.004	0.310	0.347	0.893	10.354

3.5. ADME Assessment of Hit Compounds

The evaluation of pharmacological and physicochemical profiles is crucial in assessing the potential of a drug to reach its target site and exert a sustained biological response. In this context, physicochemical profiling was conducted using Lipinski's rule of five (Ro5) to determine the oral activity potential of the proposed molecules. Lipinski's criteria include molecular weight (Mw) ≤ 500 Da, LogP ≤ 5, hydrogen bond donors ≤ 5, hydrogen bond acceptors ≤ 10, and 40 ≤ molar refractivity ≤ 140 [7]. SwissADME [10] predicted all the hit compounds to be orally active, complying with Lipinski's rules. Furthermore, Veber's rule [44], which emphasizes the importance of a limited number of rotatable bonds (≤10) and a polar surface area (PSA) of ≤140 Å² for oral activity, was employed. The identified hit compounds were found to adhere to Veber's rule, reinforcing their potential for oral activity. Solubility, a critical factor influencing drug bioavailability, was evaluated to predict the dissolution of the hit compounds in the bloodstream [41]. A logS > -6 is recommended for the solubility of a potential drug candidate [15]. While B2 and B7 exhibited poor solubility, the remaining compounds showed moderate to high solubility. Interestingly, spironolactone and metformin, known drugs, displayed contrasting solubility characteristics, with spironolactone predicted to be soluble and clomiphene exhibiting poor solubility. An assessment of the potential of the compounds to react non-specifically with various biological targets was conducted using the PAINS (Pan-Assay Interference Compounds) filter [2]. Gastrointestinal absorption (GI) was evaluated to gauge the ability of the hit compounds to be absorbed into the intestines, with a high GI indicating complete absorption into the bloodstream [25]. The hit compounds showed a high GI, suggesting their potential drug-likeness, although clomiphene was predicted to have low GI, and spironolactone and metformin exhibited

different characteristics. Molar refractivity (MR) was assessed to provide crucial information on the pharmacokinetics and pharmacodynamics of the selected hit compounds [36]. The overall polarity of the hit compounds, except for B2 and B7, fell within the acceptable MR range of less than 130 Å². Synthetic accessibility (SA), an indicator of how feasible it is to synthesize a drug, was evaluated, with lower SA values being desirable [12]. All the hit compounds, as well as the known drugs, were found to have SA values lower than 6. The ability to cross the blood-brain barrier (BBB) was examined, with B4 being the only predicted hit capable of permeating the BBB and attaching to brain parenchyma and activating certain receptors. This contrasted with the controls, which did not exhibit this capability. Moreover, the prediction of P-glycoprotein (P-gp) substrate status, a factor influencing bioavailability, revealed that B2 and B7, along with metformin and spironolactone, were not predicted to be P-gp substrates. In contrast, the remaining five hits, including clomiphene, were predicted to be P-gp substrates. In summary, the results from the comprehensive pharmacological and physicochemical profiling underscore the potential of the de novo-designed ligands to be considered druggable candidates, with favourable attributes for further development and exploration.

Table 3. *Pharmacological and physicochemical profiling of the hit compounds computed molecular weight (MW), number of heavy atoms (NHA), molar refractivity (MR), topological polar surface area (TPSA), gastrointestinal absorption (GI), blood-brain barrier (BBB), Gerber's violations, synesthetic accessibility (SA), and P-glycoprotein (P-gp) substrate status.*

Compound	Mw (g/mol)	NHA	MR	TPSA (A^2)	ClogP	LogS	PAINS ALERT	GI	Lipinski violations	Veber violations	SA	BBB	P-gp
B1	427	28	115.62	94.94	3.481	Moderate	0	High	0	0	5.68	No	Yes
B2	457.56	34	134.23	80.14	2.2186	Poor	0	High	0	0	5.3	No	No
B3	392.56	27	110.73	103.73	1.6575	Moderate	0	High	0	0	5.57	No	Yes
B4	408.53	30	122.07	71.33	3.1706	Moderate	0	High	0	0	5.6	Yes	Yes
B5	372.46	27	102.51	103.02	4.4829	Soluble	0	High	0	0	4.59	No	Yes
B6	370.53	27	121.78	65.19	3.003	Moderate	0	High	0	0	5.83	No	Yes
B7	472.66	34	142.91	86.45	6.1754	Poor	0	High	1	0	5.83	No	No
Metformin	129.16	9	36.93	88.99	-1.7137	soluble	0	High	0	0	3.11	No	No
Clomiphene	405.96	29	124.52	12.47	5.1364	Poor	0	Low	1	0	3.04	No	Yes
Spironolactone	416.57	29	115.23	85.74	3.2098	Moderate	0	High	0	0	5.94	No	No

3.6. Toxicity Profiling

The toxicity assessment of the seven compounds was meticulously conducted using OSIRIS Data Warrior 5.0.0 and ProTox II, reflecting a comprehensive approach to understanding potential risks associated with these compounds in the drug development process. The significance of toxicity assessments in drug design has grown immensely, considering that numerous drugs have faced setbacks in reaching clinical trials due to safety concerns [42]. In some instances, drugs already in use have been subject to recalls due to reported toxicity issues [28]. Recognizing the hazards associated with drugs exhibiting various levels of toxicity has emphasized the importance of employing multiple in silico models in drug risk assessment [33]. The toxicity profiling considered for this study encompassed mutagenicity, tumorigenicity, hepatotoxicity, immunotoxicity, cytotoxicity, reproductive effects, and irritants. Mutagenicity, the potential of a drug to alter the DNA of an organism, is a critical consideration, and all selected hits (Table 4) were predicted not to be mutagenic. Immunotoxicity, involving adverse modulation of the immune system, is another crucial aspect, especially considering the therapeutic agents that modulate the host immune system for the treatment of human ailments [17]. All proposed hits were demonstrated to be non-immunotoxin, in contrast to spironolactone and clomiphene, which exhibited immunotoxin effects. Tumorigenicity, the ability of a drug to induce or cause proliferation of cancer cells, is a serious concern. However, the identified hit compounds were predicted not to possess any tumorigenic effects. Reproductive effects, which interfere with the ability to produce normal, healthy offspring, were not indicated for any of the proposed hits, highlighting their safety in this regard. Cytotoxicity, the harm caused by chemotherapeutic agents on living cells, is a vital consideration, especially given the application of cytotoxic compounds in the treatment of diseases like cancer [50]. Interestingly, all compounds, except B5 (Table 4), were predicted to have low cytotoxicity properties. Hepatotoxicity, a life-threatening impairment of liver function caused by exposure to xenobiotics, was assessed, and the predictions indicated that all hit compounds, except for B6, were inactive about hepatotoxic effects. In summary, the overall predictions from these toxicity assessments suggest a safe pharmacokinetic and pharmacodynamic profile for all the molecules, underscoring the need for further investigations and supporting their potential as candidates for continued development.

Table 4. *Toxicity profiling of hit compounds.*

Compound	Mutagenic	Irritant	Tumorigenic	Reproductive effect	Hepatotoxicity	Carcinogenic	Cytotoxic	Immunotoxin
B1	NONE	NONE	NONE	NONE	Inactive	Inactive	Inactive	Active
B2	NONE	NONE	NONE	NONE	Inactive	Inactive	Inactive	Inactive
B3	NONE	NONE	NONE	NONE	Inactive	Active	Inactive	Inactive

Compound	Mutagenic	Irritant	Tumorigenic	Reproductive effect	Hepatotoxicity	Carcinogenic	Cytotoxic	Immunotoxin
B4	NONE	NONE	NONE	NONE	Inactive	Inactive	Inactive	Active
B5	NONE	NONE	NONE	NONE	Inactive	Inactive	Active	Active
B6	NONE	NONE	NONE	NONE	Active	Inactive	Inactive	Active
B7	NONE	NONE	NONE	NONE	Inactive	Inactive	Inactive	Active

3.7. Biological Activity Predictions

The Open Bayesian machine learning algorithm, Prediction of Activity Spectra for Substances (PASS) [24], derived from a structure-activity relationship (SAR) analysis of chemical entities in the database [29], was employed to anticipate the biological activity of selected molecules (Table 4). According to PASS predictions, a ligand exhibiting a probability of activity (Pa) greater than the probability of inactivity (Pi) implies the compound's potential for biological activity, warranting experimental validation [5]. All compounds, excluding B3, were projected as CYP17 inhibitors with Pa values of 0.348, 0.383, 0.334, 0.435, 0.432, and 0.533, and Pi values of 0.065, 0.050, 0.037, 0.034, 0.035, and 0.016, respectively. Abiraterone, an oral medication inhibiting both 17-alpha hydroxylase and 17,20-lyase activities of CYP17A1 [27], disrupts the conversion of pregnenolone and progesterone, potentially linked to PCOS. This corroborates the biological activity of the predicted compounds. Additionally, all seven compounds were forecasted as potential prostate disorder treatment drugs with Pa values of 0.330, 0.174, 0.398, 0.459, 0.220, 0.496, and 0.430, all surpassing the respective Pi values of 0.060, 0.036, 0.040, 0.027, 0.028, 0.021, and 0.032. Comparative analysis with Spironolactone and Cyproterone acetate, prostate disorder treatment drugs that decrease the activity of 5 alpha-reductase 2 enzymes and have been employed in PCOS treatment [18], suggests the potential utility of our hits in PCOS treatment.

Table 5. *Biological predictions of selected hit compounds.*

Compound	CYP 17 Inhibitor		Contraceptive		17 alpha-hydroxylase Inhibitor		Anti-infertility		CYP2C9 Inducer		Menstrual disorder treatment		Prostate disorder treatment	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
B1	0.348	0.065	-	-	-	-	0.169	0.148	-	-	-	-	0.330	0.060
B2	0.383	0.050	0.187	0.045	-	-	-	-	0.299	0.146	0.162	0.112	0.174	0.036
B3	-	-	-	-	-	-	0.312	0.043	-	-	-	-	0.398	0.040
B4	0.334	0.037	0.272	0.209	-	-	-	-	-	-	-	-	0.459	0.027
B5	0.435	0.034	0.305	0.017	-	-	-	-	0.567	0.007	-	-	0.220	0.028
B6	0.432	0.035	0.141	0.116	0.072	0.047	-	-	0.397	0.047	0.154	0.128	0.496	0.021
B7	0.533	0.016	0.409	0.011	0.091	0.030	-	-	0.264	0.224	-	-	0.430	0.032

Furthermore, compounds B2, B4, B5, B6, and B7 were predicted as contraceptive drugs. A previous study highlighted the effectiveness of using oral contraceptives in the treatment of PCOS [21], this, then again solidifies the potential activity of the hit compounds. Notably, B2 showed Pa (0.187) and Pi (0.045), and B6 and B7 were identified as 17 alpha-hydroxylase inhibitors, reinforcing their potential in potential activity of the hit compounds. Notably, B2 showed Pa (0.187) and Pi (0.045), and B6 and B7 were identified as 17 alpha-hydroxylase inhibitors, reinforcing their potential in PCOS treatment. Compounds B2 and B6, with Pa values of 0.162 and 0.154, and Pi values of 0.0112 and 0.128, were also projected as menstrual disorder treatment drugs. This aligns with previous findings on Metformin, a menstrual and insulin-regulatory drug used in PCOS treatment [45]. In conclusion, the amalgamation of biological activity predictions and exploration of compounds in the DrugBank PCOS treatment. Compounds B2 and B6, with Pa values of 0.162 and 0.154, and Pi values of 0.0112 and 0.128, were also projected as menstrual disorder treatment drugs. This aligns with previous findings on Metformin, a menstrual and insulin-regulatory drug used in PCOS treatment [45]. In conclusion, the amalgamation of biological activity predictions and exploration of compounds in the DrugBank suggests the potential of de novo-designed compounds as promising lead molecules necessitating experimental validation.

4. Conclusion

This study employed an integrated in silico approach to identify novel non-steroidal inhibitors of cytochrome P450c 17 alpha for the potential treatment of polycystic ovary syndrome. The integrated computational approach employed in this study led to the identification of seven novel non-steroidal scaffolds as potential CYP17A1 inhibitors for PCOS treatment. The favourable drug-like properties, interactions, binding affinities and biological activity predictions of the hits warrant further experimental validation to explore their therapeutic potential against this complex endocrine disorder.

Author Contributions

G. D. and C. A. B. conceptualized the project idea. G. D, P. Y, K. G and G. D. executed all the computational analyses with meaningful contributions from C. A. B, G. D, P. Y, K. G, J. A. A and S. A. R wrote the first draft after which all the authors read, made their inputs, and finally accepted by all

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Data Availability Statement

The data supporting the current study are available from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflicts of interest.

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