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Molecular Modelling of Withanolides Against EP2 Receptor for Treatment of Endometrial Cancer: A Pharmacokinetic and Molecular Docking Study †

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Abstract

Endometrial cancer is one of the most common gynecological cancers, with global new cases, approximately 420,000 new cases and 98,000 global deaths annually. Emerging evidence suggests that the EP2 plays a critical role in tumor progression, angiogenesis, and immune evasion. Withanolides, a class of naturally occurring compounds, possess anticancer activity; however, effect on EP2 receptor in endometrial cancer remains largely unexplored. This study aims to explore the interaction between Withanolides and EP2 receptor using molecular docking techniques, with PF-04418948 as the reference antagonist. A select number of these ligands, with anti-cancer activity, were evaluated for their ADMET properties, and those with favorable drug-like properties, low toxicity profile, and no more than 1 Lipinski's rule violation, were docked to EP2 (PDB ID: 7CX2). Molecular docking studies revealed three ligands, (Pubchem 161671, 265237, and 21679027) with significantly higher binding affinity scores compared to that of the reference compound. Pubchem 161671, showed the highest binding affinity at −12.6 kcal/mol. Post-dock analysis revealed interactions with key amino acids, VAL89, LEU298, SER305, and MET31, which are essential for the antagonist activity of the EP2 receptor enzyme [1]. Significant interaction with critical amino acid residues suggested potential inhibition of EP2 receptor activity, offering a potential therapeutic approach for treating endometrial cancer. Overall, this study profers a deeper understanding of the potential of Withanolides as leads for EP2 targeted therapy in endometrial cancer.

Keywords: endometrial cancer; prostaglandin E2 receptor (EP2); withanolides; molecular docking; ADMET; anticancer activity; targeted therapy

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1. Introduction

Endometrial cancer (EC) is one of the leading gynecological malignancies worldwide, and its burden is steadily rising in both developed and developing regions. In Nigeria and across sub-Saharan Africa, challenges such as poor cancer registries, delayed presentation, and limited access to diagnostic and treatment facilities contribute to poorer outcomes compared with high-income settings [2–4]. Clinical reports from Nigerian tertiary hospitals describe EC as a common genital tract malignancy, often presenting with

abnormal uterine bleeding, pelvic discomfort, or postmenopausal bleeding, with many patients already at advanced stages when diagnosed [5,6]. Biologically, EC is a heterogeneous disease driven by mutations in genes such as PTEN, PIK3CA, ARID1A, and TP53, and by activation of pathways including PI3K/AKT/mTOR and Wnt/β-catenin [3]. Increasing attention has also turned to PGE2 signaling, particularly via the EP2 receptor, a G protein–coupled receptor that enhances tumor growth, angiogenesis, metastasis, and immune evasion [7–9]. While surgery, radiotherapy, chemotherapy, and immunotherapy remain standard treatments, recurrence, systemic toxicity, and limited responses in unselected patients highlight the need for novel targeted therapies [10–13]. Withanolides, naturally occurring steroidal lactones from Withania somnifera (ashwagandha) and other Solanaceae species, are well known in traditional medicine and widely distributed across South Asia, the Middle East, Africa, and the Mediterranean [14]. They have attracted growing interest for their broad pharmacological properties, including anti-inflammatory, immunomodulatory, and anticancer effects [15]. In experimental cancer models, withanolides such as withaferin A suppress tumor growth, trigger apoptosis, block angiogenesis, and interfere with oncogenic signaling pathways including NF-κB and PI3K/AKT [16–18]. Although their direct interaction with prostaglandin receptors has not been fully established, recent computational and biochemical studies suggest that withanolides may interact with G protein–coupled receptors, making them promising candidates for further exploration against EP2 [17,19].

This study applies a structure-based in silico approach to evaluate natural and semi-synthetic withanolides as potential EP2 receptor antagonists in EC. By combining molecular docking, binding analysis, molecular dynamics refinement, and ADMET screening, we aim to identify withanolide scaffolds with high affinity and favorable drug-like properties. Computational methods such as molecular docking offer cost-effective and reliable ways to predict ligand–receptor interactions and guide early-stage drug discovery [20–22]. The identification of potent withanolide-EP2 interactions could pave the way for novel therapeutic options that inhibit PGE2-driven tumor progression, with particular relevance for resource-limited settings where EC outcomes remain poor.

2. Materials and Methods

2.1. Software, Hardware, and Databases

AutoDock Vina version 1.5.6 [23], UCSF Chimera [24], BioviaDiscovery Studio 2021 [25], Spartan 04, ChemSpider [26], SwissAdme [27], PROTOX III [28], Windows (Intel processor, Corei7, 4gb RAM).

Protein Crystal Structure

High-resolution, Cryo-EM structure of the Human prostaglandin E2 receptor was obtained from RCSB Protein Data Bank (http://www.rcsb.org/pdb) PDB ID: 7CX2] [29].

2.2. In Silico Anticancer Studies

2.2.1. Evaluation of Theoretical Oral Bioavailability

The oral bioavailability of the selected compounds lig1, lig2 and lig3 was predicted theoretically based on Lipinski's rule of five. The SWISSADME server [27], and PROTOX-III [28] servers were used for properties that defined the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the compounds, respectively. The servers also predicted various physicochemical properties including lipophilicity, water solubility, drug-likeness, medicinal attributes, and compound toxicity with high precision.

2.2.2. Protein Structure Preparation

In this study, the protein Cryo-EM structure of the PGE2-bound EP2-Gs complex with 7CX2 code and Electron microscopy at 2.80 Å was downloaded from Protein Data bank and was used as the receptor starting structure. All crystallographic water molecules, ions and bound ligands were removed from the 3-D structures obtained from the protein data bank using Chimera UCSF [24]. The crystallized ligands were separated and prepared using Chimera, then saved as lig.mol, while the receptors were isolated and prepared, then saved as rec.pdb. The output files from chimera were then inputted into Autodock tools, where lig.mol and rec.pdb were edited by adding polar hydrogen and Gasteiger charges before being saved as pdbqt files [30]. AutoDock tools were applied in order to help set up docking runs and predict binding free energy of the test ligands.

2.2.3. Ligand Structure Preparation

The 2D structures of the characterized compounds lig1, lig2, lig3 and the reference ligand, PF-04418948 was downloaded from Chemspider server, and Spartan 04 was used to convert the 2D structures to 3D. Using the AMI semi-empirical method, geometrical optimization was carried out on all the compounds using the Spartan software version 1.1.4, and the optimized structures were stored as mol2 files. AutoDock Tools was used to add hydrogen and Gastegier charges and saved as mol2 files to pdbqt format [24].

2.2.4. Molecular Docking Analysis

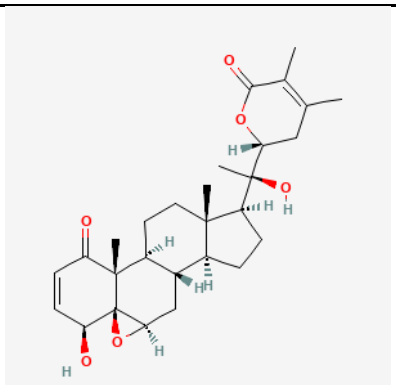
Dock validation was carried out before docking of the test compounds. The co-crystallized ligand was separated from the enzyme cryo structure and re-docked using the set-up grid parameters. When a conformation, superimposable with a geometrical conformation of the co-crystallized ligand in the active site was achieved, this grid parameter was used to dock the test compounds. Before molecular docking, the active sites were defined according to the coordinates of the crystallographic structures of both enzymes by defining the grid box, and the best pose was obtained, which was used for further studies. The UCSF Chimera was further used for post-docking visualization.

3. Results

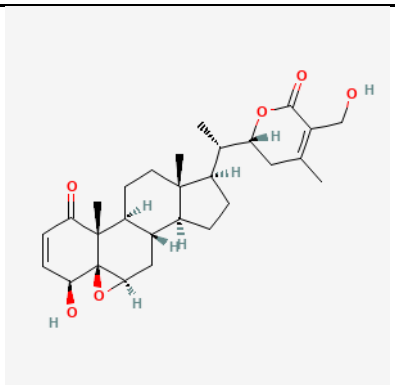
3.1. Creation of Library

Table 1 shows the selected Withanolides. Three compounds from Withanolides class were downloaded from Chemspider.

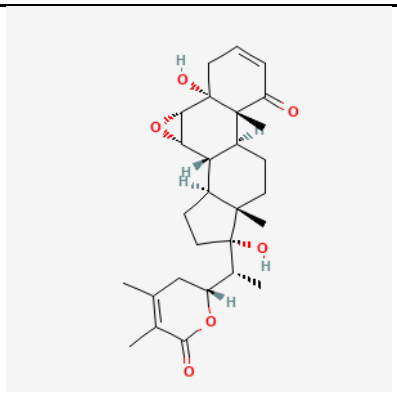
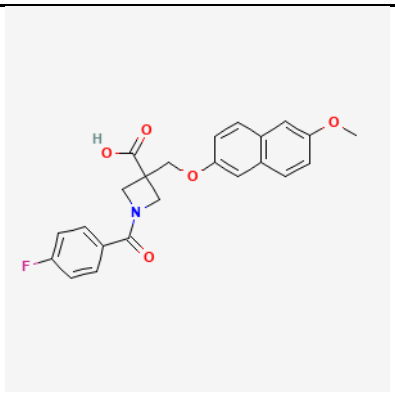
Table 1. Library of Withanolides derivatives.



Lig 1(Withanolide D)



Lig 2(Withaferin A)

	
Lig 3 (Withanone)	PF-04418948

3.2. Analysis of Theoretical Oral Bioavailability

Table 2 displays the calculated theoretical oral bioavailability parameters of all five selected compounds. The drug-likeness of these compounds was assessed based on the Lipinski's rule of five i.e., their molecular weight (≤500 Da), hydrogen bond donors (≤5), hydrogen bond acceptors (≤10), and their MlogP values (≤5) [31]. Additional parameters such as: The Topological Polar Surface Area (TPSA), number of rotatable bonds and Molar Refractivity (MR), were also evaluated in the context of Veber's rule, Egan's rule, Ghose's rule and Muegge's rule.

Table 2. Analysis of theoretical oral bioavailability of selected compounds based on Lipinski's rule of five and pharmacokinetic parameters.

Properties	**lig1**	**lig2**	**lig3**
CID	161671	265237	21679027
Molecular Formula	$C_{28}H_{38}O_6$	$C_{28}H_{38}O_7$	$C_{28}H_{38}O_6$
Mol. Wt (g/mol)	470.60	486.60	470.60
Heavy atoms	34	35	34
Aromatic Heavy atoms	0	0	0
Fraction Csp3	0.79	0.79	0.79
HbA	6	7	6
HbD	2	3	2
Nrb	2	3	2
MR	127.53	128.69	127.53
TPSA (Å^2)	96.36	116.59	96.36
MlogP	2.75	1.95	2.75
Lipinski violation	No	No	No
Inference	Pass	Pass	Pass
Ghose violations	1	2	1
Veber violations	0	0	0
Egan violations	0	0	0
Muegge violations	0	0	0
Bioavailability score	0.55	0.55	0.55
Synthetic accessibility	6.85	6.88	6.38

ADMET Profile

Table 3 shows the water solubility values, expressed as log S_w. Swissadme server calculated values ranging from −3.20 to −4.00, indicating that the selected compounds possess good water solubility. The CytochromeP450 inhibitory potential of the compounds is also shown in the table. Lipophilicity values for the compounds, expressed as Consensus $LogP_{o/w}$ ranged from 2.64 to 3.39. Table 3 shows the toxicity profile test of the compounds.

Table 3. Pharmacokinetics prediction output and oral bioavailability of lig1–lig3 compounds.

Properties	Lig1	Lig2	Lig3
LogS (Silicos-IT)	−3.78	−3.20	−4.00
Silicos-class	Moderately soluble	Moderately soluble	Moderately soluble
Consensus Log $P_{o/w}$	3.39	2.64	3.36
Log Kp (skin permeation) (cm/s)	−6.96	−7.55	−7.01
GI Absorption	High	High	High
BBB Permeant	No	No	No
Pgp substrate	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No

Table 4. Toxicity profile of the test compounds.

Properties	Lig1	Lig2	Lig3
Oral acute toxicity	Class III	Class III	Class II
Ames Mutagenesis	-	-	-
Carcinogenicity	-	-	-
Hepatotoxicity	-	-	-
Androgen receptor binding	-	-	-
Thyroid receptor binding	-	-	-
Estrogen receptor binding	-	-	-
Aromatase binding	-	-	-

-: Inactive; Class II: 5 mg/kg ≤ LD_{50} ≤ 50 mg/kg; Class III: 50 mg/kg ≤ LD_{50} ≤ 300 mg/kg;.

3.3. Molecular Docking Studies

3.3.1. Grid Box

The gridbox parameter, was used to generate the configuration file(config.txt). AutoDockVina produced results in pdbqt format, with the compounds saved in complexes alongside the reference enzymes. The specific gridbox parameter is detailed in Table 5 below.

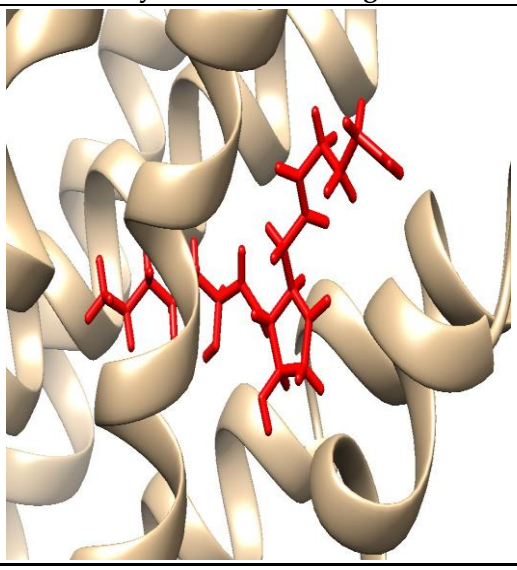
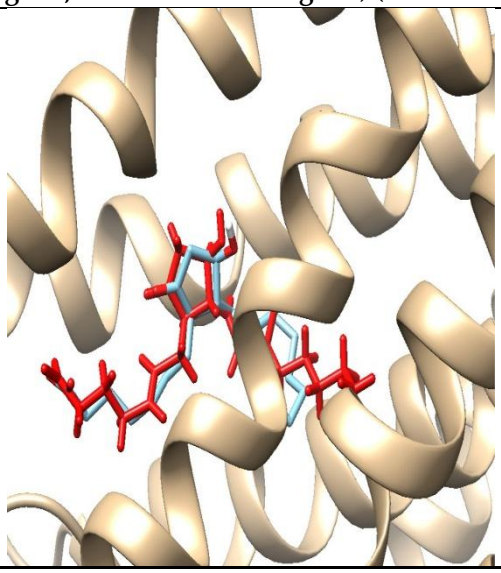
Table 5. Enzyme Grid-box Size Center.

ENZYME	GRID-BOX SIZE			CENTER		
	X	Y	Z	X	Y	Z
7CX2	22	16	22	3.719	114.893	119.227

3.3.2. Validation of Docking Procedures

Validation of the docking procedure is shown in Table 6. The protein structure was downloaded from Protein Databank (PDB) with a Co-crystallized molecule superimposed. In the dock validation Procedure, the co-crystallized molecule was re-docked on the receptor.

Table 6. Crystal structure of bio-target complex and re-docked ligand super-imposed on the crystal structure for validation.

Enzyme Code and Name	Crystal Structure Complex (Enzyme and Native Ligand)	Crystal Structure Complex (Enzyme, Native Ligand, and Re-Docked Ligand) (Validation)
7CX2 PGE2-bound EP2-Gs complex		

3.3.3. Binding Affinity Ligands to EP2 Enzyme

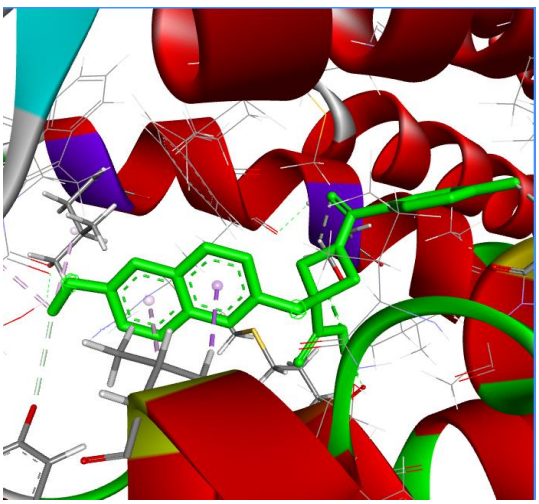
Table 7 shows the binding energies of both the reference compound and the three selected compounds in their interactions with the EP2 enzyme.

Table 7. The binding energies of the reference compound and the three selected compounds against EP2 receptor.

Enzyme	Affinity (kcal/mol)			
	PF-04418948	Lig1	Lig2	Lig3
7CX2	−9.6	−12.6	−12.0	−11.3

3.3.4. Binding Poses and Binding Interaction Analysis of Isolated Compounds Against EP2 Enzyme

The binding conformation and interaction with residues on the active site of the enzymes studied using Chimera (Pettersen et al., 2004) and Discovery studio visualizer (Free Download: BIOVIA Discovery Studio Visualizer—Dassault Systèmes, n.d.) are shown on 1-



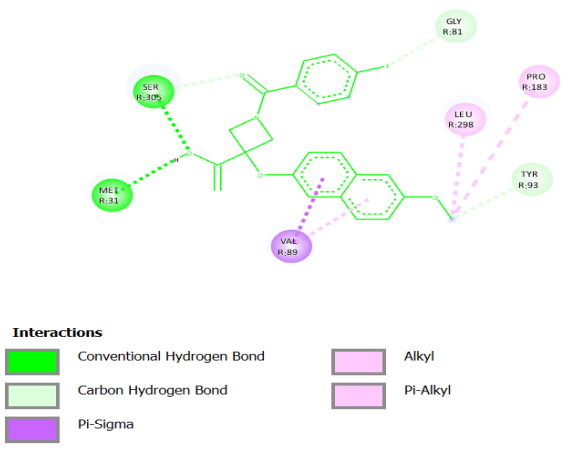


Figure 1. Three-dimensional molecular pose (**left**) and 2D (**right**) interactions of PF-04418948 on binding cavity of EP2 enzyme.

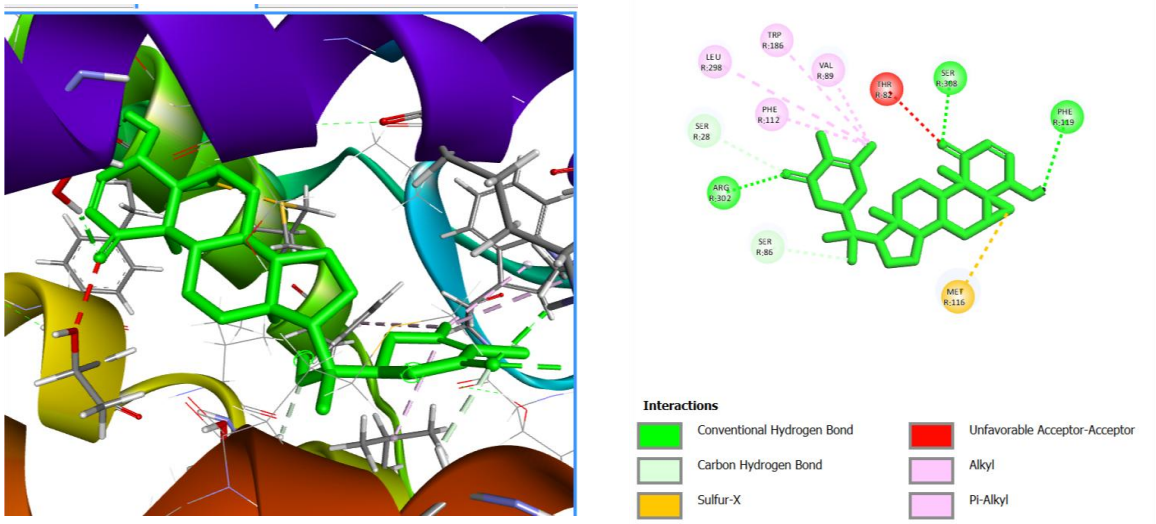


Figure 2. Three-dimensional molecular pose (**left**) and 2D (**right**) interactions of lig1 on binding cavity of EP2 enzyme.

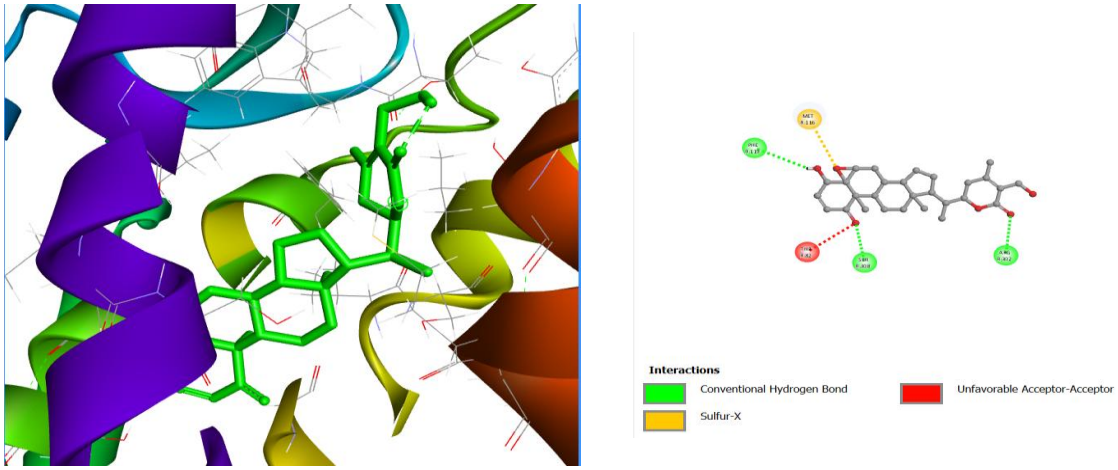


Figure 3. Three-dimensional molecular pose (**left**) and 2D (**right**) interactions of lig2 on binding cavity of EP2 enzyme.

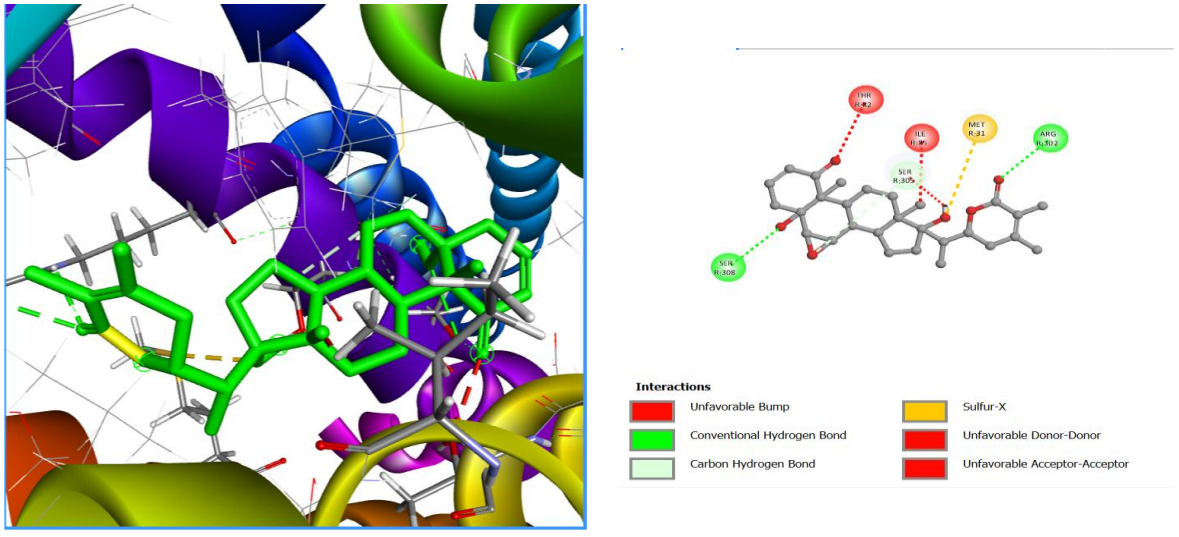


Figure 4. Three-dimensional molecular pose (**left**) and 2D (**right**) interactions of lig3 on binding cavity of EP2 enzyme.

Table 8. Molecular interactions of amino acid residues of withanolide compounds with EP2 enzyme.

Compounds	Interaction Type	Interacting Residues
PF-04418948	Conventional Hydrogen Bond	SER305, MET31
	Alkyl	LEU298, PRO183
	Pi-Alkyl	VAL89
	Carbon Hydrogen Bond	TYR93, GLY81
Lig 1	Pi-Alkyl	PHE112, TRP186
	Alkyl	VAL89, LEU298
	Conventional Hydrogen Bond	ARG302, SER308, PHE119
	Sulphur-X	MET116
	Carbon Hydrogen Bond	SER28, SER86
Lig 2	Conventional Hydrogen bond	SER305, ARG302
	Sulfur-X	MET31
	Carbon Hydrogen Bond	SER308
Lig3	Conventional hydrogen bond	ARG302, SER302
	Carbon Hydrogen Bond	SER305, SER308
	Sulfur-X	Met31

4. Discussion

The results of this study, show that withanolides could be promising candidates for targeting the EP2 receptor in endometrial cancer. Looking at their drug-like properties, all three compounds: Withanolide D (lig1), Withaferin A (lig2), and Withanone (lig3), performed well. Their molecular weights were within the drug-likeness threshold (470–486 g/mol), and they had acceptable numbers of hydrogen bond donors and acceptors. The lipophilicity values (MlogP 2.75, 1.95, and 2.75) suggest that these compounds are moderately hydrophobic, which is a sweet spot for oral absorption. Interestingly, lig2 was slightly more polar, which may reduce passive diffusion but could improve solubility. All three scored 0.55 for oral bioavailability, pointing to a good chance of achieving effective blood levels if developed as oral drugs. The ADMET screening gave more encouraging insights.

Their lipophilicity scores (LogP 2.64–3.39) fall into the optimal range for drug molecules. Each showed high gastrointestinal absorption, no penetration across the blood–brain barrier, and importantly, no inhibition of cytochrome P450 enzymes—meaning fewer chances of drug–drug interactions. Solubility was moderate (LogS −3.20 to −4.00), which is not unusual for natural compounds and could be improved with formulation strategies. Overall, these pharmacokinetic features line up with what is typically seen in successful natural product-based drugs.

Toxicity analysis showed some disparities. Lig2 (Withaferin A) was classified as toxicity Class II, meaning it can be fatal if swallowed at high doses, while lig1 and lig3 were in Class III, which is still toxic but less severe. While this indicates a red flag, it is important to note that, many anticancer agents fall into similar categories; patient-dependent dose management is crucial. None of the compounds were predicted to be mutagenic, carcinogenic, or hormonally disruptive, which is a major strength for further development.

The docking studies revealed that the reference drug PF-04418948 bound to the EP2 receptor with an affinity of −9.6 kcal/mol, but all three withanolides bound much more strongly: −12.6 kcal/mol for lig1, −12.0 for lig2, and −11.3 for lig3. These higher scores

suggest that withanolides may be even more effective inhibitors than the known antagonist. A closer look at the binding sites, revelaed that the compounds were found to interact with key residues—especially SER305 and MET31—that are critical for blocking EP2 activity [1]. Lig1, in particular, showed multiple stabilizing interactions, which may explain its best performance.

5. Conclusions

Altogether, these findings suggest that withanolides—especially Withanolide D—strike a valuable balance of drug-likeness, manageable toxicity, and strong inhibitory potential against the EP2 receptor. Since EP2 is implicated in tumor progression, angiogenesis, and immune evasion, blocking it could open up new therapeutic avenues for endometrial cancer. While laboratory and clinical studies are still needed to validate these results, our computational approach offers an early but strong case for withanolides as affordable and effective candidates for targeted cancer therapy.

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Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest:

Abbreviations

The following abbreviations are used in this manuscript:

EP2	Prostaglandin E2 receptor subtype EP2
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity.
PDB ID	Protein Data Bank Identifier
EC	Endometrial Cancer
PTEN	Phosphatase and TENsin homolog
PIK3CA	Phosphatidylinositol-4,5-biphosphate 3-Kinase Catalytic Subunit Alpha
ARID1A	AT-rich Interaction Domain-containing Protein 1A
TP53	Tumor Protein 53
PI3K/AKT/Mtor	Phosphatidylinositol-3-kinase/Protein kinase B/Mammalian Target of rapamycin
NF-Kb	Nuclear Factor kappa-light-chain-enhancer of activated B cells
UCSF	University of California, San Franscisco
PDBQT	Protein Data Bank, Quaternion, Torsion

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