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Molecular docking of selected steroid compounds from *Hydrilla Verticillata* on human Ros1 kinase receptor

Molecular docking of selected steroid compounds from *Hydrilla Verticillata* on human Ros1 kinase receptor

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Abstract. *Hydrilla verticillata*, an aquatic plant, contains various steroids like stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol with potential antioxidant properties. Antioxidants protect cells and tissues from Reactive Oxygen Species (ROS)-induced damage by impeding oxidation reactions and ROS elimination. This study aimed to investigate the molecular docking results of selected steroid compounds from *H. verticillata* with the human ROS1 kinase receptor. Five selected steroid compounds, collected from the PubChem database. The human ROS1 kinase receptor, PDB ID 3ZBF, was obtained from the RCSB Protein Data Bank. Molecular docking of these selected steroid compounds to human ROS1 Kinase was performed, comparing their binding affinities to crizotinib (native ligand) and ascorbic acid. The docking utilized the PyRx Virtual Screening Tool and visualization via BIOVIA Discovery Studio Visualizer. Results indicated that stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol had binding affinities of -8.1, -8.2, -8.6, -8.4, and -8.5 kcal/mol, respectively, to the 3ZBF human ROS1 kinase receptor. While, crizotinib and ascorbic acid exhibited binding affinities of -8.4 kcal/mol and -4.7 kcal/mol. Some *H. verticillata* steroid compounds displayed stronger binding affinities than crizotinib and ascorbic acid. Furthermore, these compounds complied with Lipinski's and Veber's rules and achieved bioavailability score of 0.55, suggesting their potential as antioxidants and anticancers.

Keywords: *Hydrilla verticillata*, Steroids, ROS1 kinase, antioxidant, anticancer.

1. Introduction

Cellular damage caused by reactive oxygen species (ROS), which are highly reactive oxygen-derived oxidizing compounds, plays a crucial role as free radical scavengers in the body [1]. ROS (Reactive Oxygen Species) are highly reactive oxygen-derived oxidizing compounds that can cause DNA mutations and, as a result, trigger the development of cancer [2]. ROS (Reactive Oxygen Species) is a highly reactive chemical compound due to its ability to accept electrons from O_2 molecules [3], resulting in unstable molecules such as superoxide anion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\bullet$), and singlet oxygen ($1O^{2-}$) [4]. All types of cells produce these compounds at constant levels necessary for normal cell function and cellular balance regulation. However, if their production is excessive or cellular defenses cannot regulate them, oxidative stress (OS) can cause cellular damage [5]. The formation and effects of reactive oxygen species (ROS) in biological processes, both in terms of changes and their roles in cellular signaling and regulatory pathways, have been extensively studied. While ROS are essential components of several biological processes, such as the immune system's involvement in phagocytosis (cellular ingestion), they can also be harmful substances if they accumulate excessively in the body. Imbalance between ROS production and detoxification can lead to oxidative stress, which can damage cells and biomolecules such as DNA, proteins, and lipids. Oxidative stress has been associated with various diseases, including cancer, heart disease, and premature aging [6].

ROS-1 is one of the receptor tyrosine kinases that are pathologically and oncogenically expressed in various types of cancer [7]. Receptor tyrosine kinases (RTKs) play a pivotal role as signaling pathways for extracellular signals that regulate cell growth and survival. Uncontrolled RTK activation, caused by chromosomal rearrangements, point mutations, and gene amplification, has been established as a contributing factor in the initiation and progression of various types of cancer [8]. ROS1 kinases have been identified in diverse human cancer types and have emerged as appealing targets in the development of cancer therapies. ROS1 is a receptor with a kinase domain that is phylogenetically related to the anaplastic lymphoma kinase/lymphocyte-specific protein tyrosine kinase (ALK/LTK) and insulin receptor (INSR) tyrosine kinase families [9]. However, clinical evidence suggests that some cancer patients with ROS1 fusions, treated with crizotinib, develop mutations in the ROS1 kinase domain that lead to drug resistance [10-11]. Hence, there is a compelling need for the development of therapeutic agents capable of penetrating the central nervous system and overcoming resistance arising from ROS1 mutations in response to crizotinib treatment [12]. The structure-based pharmacophore method, involving computational techniques such as virtual screening, docking, and molecular dynamics simulations, was utilized in this study to search for ROS-1 inhibitors [13].

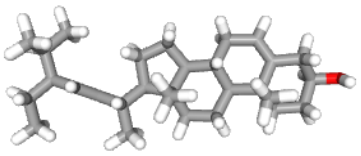
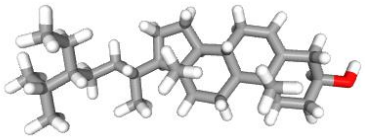
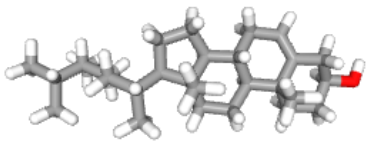
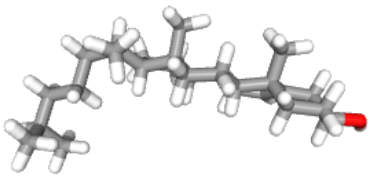
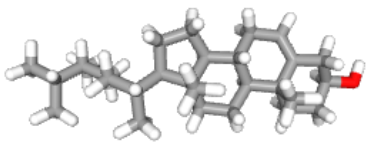
Hydrilla verticillata contains various steroid compounds, including β-sitosterol, stigmasterol, fucosterol, campesterol, and cholesterol [14]. Steroid compounds can be separated using thin-layer chromatography or column chromatography [15-17]. Some of steroid compounds from *H. verticillata* demonstrate toxicity [18-20], antioxidant [14,18,21] and potential anti-cancer properties [22]. Molecular docking results of selected phytosterol compounds from *H. verticillata* with estrogen-α and estrogen-β receptors exhibiting better binding affinities compared to genistein, used as a positive control. Stigmasterol, fucosterol, and campesterol have lower binding affinity than genistein for both estrogen-α (PDB ID: 1X7R) and estrogen-β (PDB ID: 1X7J) receptors. Among them, stigmasterol and campesterol have the lowest binding affinity for estrogen-α (PDB ID: 1X7R), while fucosterol has the lowest binding affinity for estrogen-β (PDB ID: 1X7J) receptors [22]. Meanwhile, Rosiarto et al. [1] conducted molecular docking of the compound 1-(p-chlorobenzoyloxymethyl)-5-fluorouracil on human ROS-1 kinase with PDB ID: 3ZBF, demonstrating that this compound exhibits better activity compared to ascorbic acid (Ki = -4.8 ± 0.19 kcal/mol) and 5-fluorouracil (Ki = -4.6 ± kcal/mol).

In this study, a structure-based pharmacophore approach utilizing molecular docking and pharmacoinformatics was employed to search for ROS-1 inhibitor compounds. Molecular docking analysis simulations were conducted to study the binding modes and affinities of the selested steroids compounds from *Hydrilla verticillata* within the active pocket of the ROS-1 protein. The top compounds identified through this combined pharmacoinformatics approach have the potential to serve as therapeutic agents for ROS-1-related cancer conditions.

2. Materials and Methods

The study obtained five steroid compounds from *Hydrilla verticillata* with the potential to inhibit the human ROS1 kinase receptor. These compounds, namely stigmasterol (PubChem CID: 5280794), β-sitosterol (PubChem CID: 222284), fucosterol (PubChem CID: 5281328), cholesterol (PubChem CID: 5997), and campesterol (PubChem CID: 173183) were sourced from PubChem in SDF and SMILE formats (Table 1). For comparison, crizotinib was used as the native ligand and ascorbic acid (PubChem CID: 54690394) was used as the positive control. The human ROS1 kinase receptor (PDB ID: 3ZBF) was obtained from the Protein Data Bank in PDB format. The 3-D structures were visualized using the BIOVIA Discovery Studio Visualizer (Figure 1). Molecular docking was performed using AutoDock Vina within the PyRx Virtual Screening Tool software. The grid box parameters were set to X = 35.6845; Y = 11.4127; Z = 1.5107, with dimensions (Å) at X = 49.7954, Y = 62.9755, and Z = 57.9121. The interactions between the receptor molecules and the steroid compounds were examined using BIOVIA Discovery Studio Visualizer. Physicochemical properties, pharmacokinetics, drug likeness, and bioavailability were assessed using SWISS ADME.

Table 1. The steroid compounds Compounds Identified from *Hydrilla verticillata*.

No.	Compounds	Molecule Formula	ID	Chemical Structure	SMILE
1.	Stigmasterol	$C_{29}H_{48}O$ 412.7	PubChem CID: 5280794		CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
2.	β-sitosterol	$C_{29}H_{50}O$ 414.7	PubChem CID: 222284		CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
3.	Fucosterol	$C_{29}H_{48}O$ 412.7	PubChem CID: 5281328		CC=C(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
4.	Cholesterol	$C_{27}H_{46}O$ 386.7	PubChem CID: 5997		CC(C)CCCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C
5.	Campesterol	$C_{29}H_{48}O$ 400.7	PubChem CID: 173183		CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)

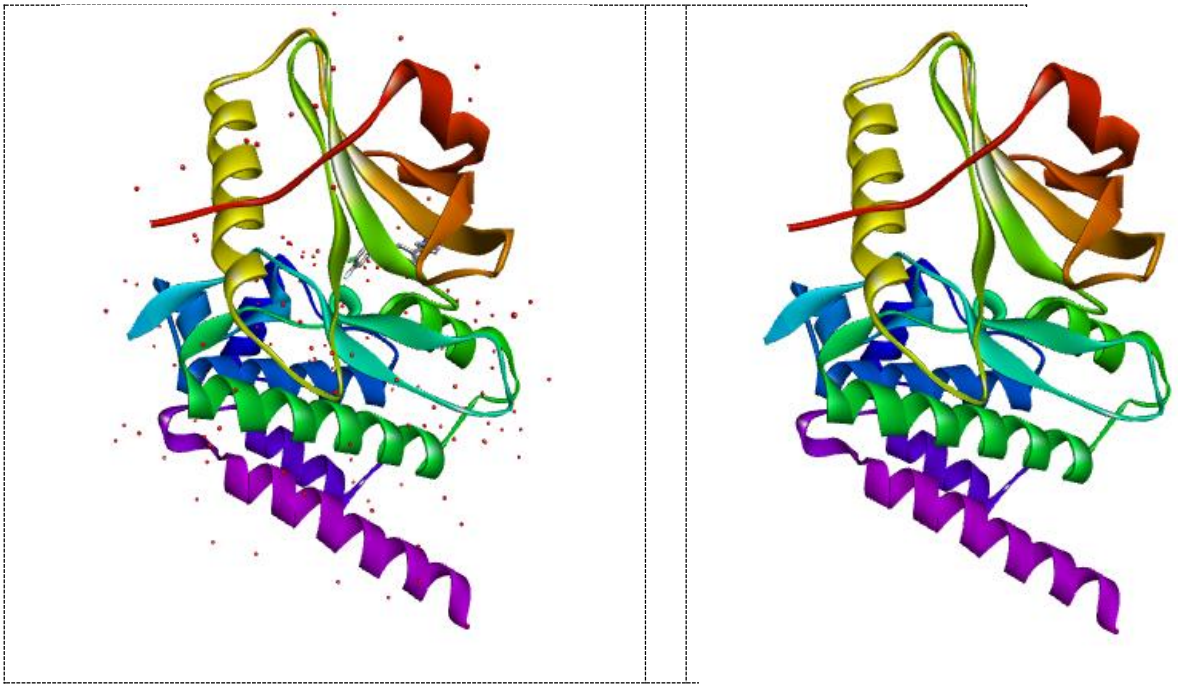


Figure 1. The human ROS1 kinase receptor (PDB ID: 3ZBF) (a), before preparation (b), after preparation

3. Results and Discussion

3.1. Docking Results Between the Human ROS1 Kinase Receptor and Selected Steroid Compounds

The docking simulations results between the Human ROS1 Kinase Receptor and stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol are outlined in Table 2 and illustrated in Figure 2.

Table 2. Docking simulation results with the Human ROS1 Kinase Receptor for selected steroid compounds from *Hydrilla verticillata*

No.	Compounds	Binding Affinity (kcal/mol)	Amino Acids residues
1.	Stigmasterol	-8.1	Leu1951, Val1959, Lys1980, Leu2026, Leu2086
2.	β-sitosterol	-8.2	Leu1951, Val1959, Lys1980, Leu2026, Leu2086, Ala1978
3.	fucosterol	-8.6	Leu1951, Val1959, Lys1980, Leu2026, Leu2086, Ala1978
4.	cholesterol	-8.4	Leu1951, Val1959, Lys1980, Leu2026, Leu2086, Ala1978
5.	campesterol	-8.5	Leu1951, Val1959, Lys1980, Leu2026, Leu2086, Ala1978
6.	crizotinib (native ligand)	-8.4	Leu1951, Val1959, Lys1980, Leu2026, Leu2086, Ala1978 Leu2010, Met2029, Asp2033, Thr2036, Asn2084
7.	Ascorbic acid	-4.7	Lys1983, Ser1986, Thr1987

The Gibbs free energy change (ΔG) is a measure of the spontaneity of a chemical reaction. A more negative ΔG indicates a more spontaneous reaction, while a less negative or positive ΔG suggests a less spontaneous or non-spontaneous reaction. The largest positive ΔG value, it would mean that the reaction is less spontaneous or more unfavorable. In such cases, the reaction may require an input of energy to proceed. Conversely, a more negative ΔG value indicates a more favorable and spontaneous reaction [23].

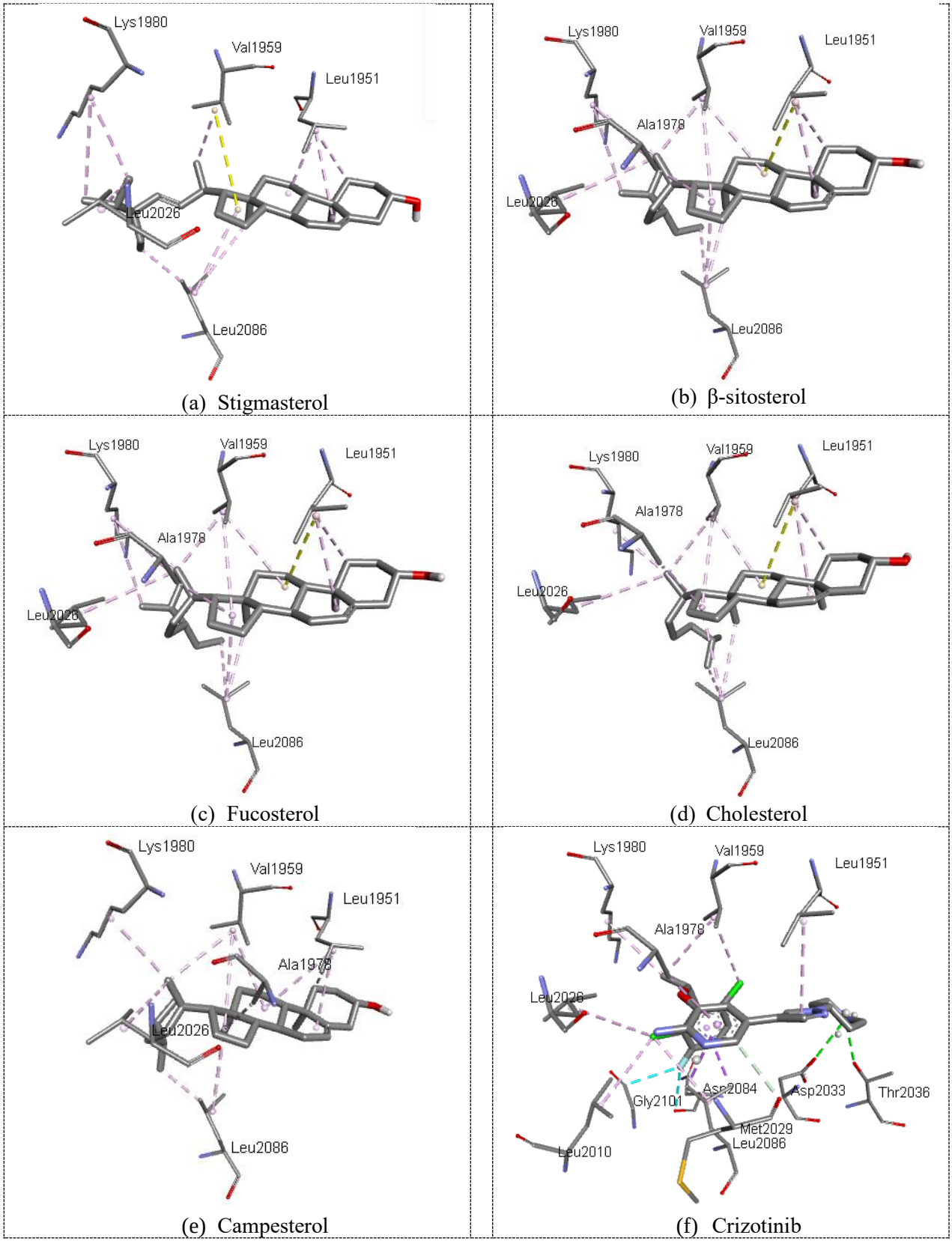


Figure 2. Ligand interactions of Selected Steroid Compounds with Human ROS1 Kinase Receptor

Stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol exhibited lower binding affinities compared to Ascorbic acid. However, when compared to the native ligand crizotinib, cholesterol showed similar binding affinity, while fucosterol and campesterol displayed lower binding affinities. Low binding affinity indicates stability and compatibility in binding to the Human ROS1 Kinase Receptor. Fucosterol, cholesterol, and campesterol show promising potential as a candidate for antioxidant and anticancer based on Human ROS1 Kinase inhibitor.

Stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol share common amino acid residues with crizotinib, including Leu1951, Val1959, Lys1980, Leu2026, and Leu2086. Additionally, β-sitosterol, fucosterol, cholesterol, and campesterol also have Ala1978 in common with crizotinib. These common residues are part of the binding site on the ROS1 kinase receptor where crizotinib binds. These residues play a crucial role in the interaction between crizotinib and the ROS1 kinase. When other compounds, such as Stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol, share these common residues, it suggests that they may have a similar binding mode or affinity for the ROS1 kinase receptor. This information is valuable in understanding the potential of these compounds as inhibitors of the ROS1 kinase. (Figure 3).

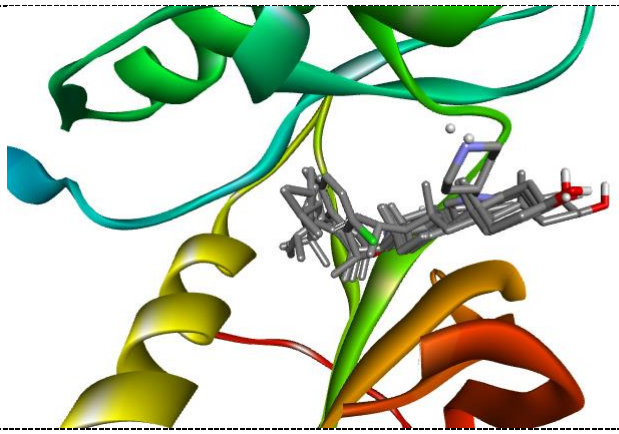


Figure 3. Positions of Stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol, and crizotinib, interact with the active site of the Human ROS1 Kinase Receptor.

The cholesterol compound form interaction with Human ROS1 Kinase Receptor 3ZBF through alkyl interaction with Leu1951 with a bond length of 3.94, 4.26, and 4.69 Å, Val1959 (4.03, 4.45, 4.92 Å), Lys1980 (3.95 Å), Leu2026 (5.25 Å), Leu2086 (3.99, 4.14, 4.69 Å), and Ala1978 with a bond length of 4.11 Å. The campesterol compound form interaction with Human ROS1 Kinase Receptor 3ZBF through alkyl interaction with Leu1951 with a bond length of 4.04, 4.27, and 4.76 Å, Val1959 (4.06, 4.41, 4.90Å), Lys1980 (3.86 Å), Leu2026 (5.24 Å), Leu2086 (4.07, 4.12, 4.72Å), and Ala1978 with a bond length of 4.10 Å. The fucosterol compound form interaction with Human ROS1 Kinase Receptor 3ZBF through alkyl interaction with Leu1951 with a bond length of 4.08, 4.33, and 4.59 Å, Val1959 (3.86, 4.51, 4.91 Å), Lys1980 (4.02 and 4.77 Å), Leu2026 (4.79 and 5.09 Å), Leu2086 (4.04, 4.61, 4.80Å), and Ala1978 with a bond length of 4.00 Å (Figure 4).

Studying the interactions between compounds and ROS1 kinase through molecular docking and other computational techniques can help identify potential inhibitors and understand how they bind to the kinase's active site. This knowledge is crucial for the development of targeted cancer therapies. Human ROS1 kinase (ROS1) is a receptor tyrosine kinase that plays a crucial role in various cellular signaling pathways. It is a member of the ROS1 proto-oncogene receptor tyrosine kinase family and is involved in regulating cell growth and survival. Aberrant activation of ROS1 kinase has been associated with the development and progression of certain types of cancer. Therefore, it has become an appealing target for cancer therapy. Inhibiting ROS1 kinase activity has shown promise as a therapeutic strategy for treating ROS1-related cancers.

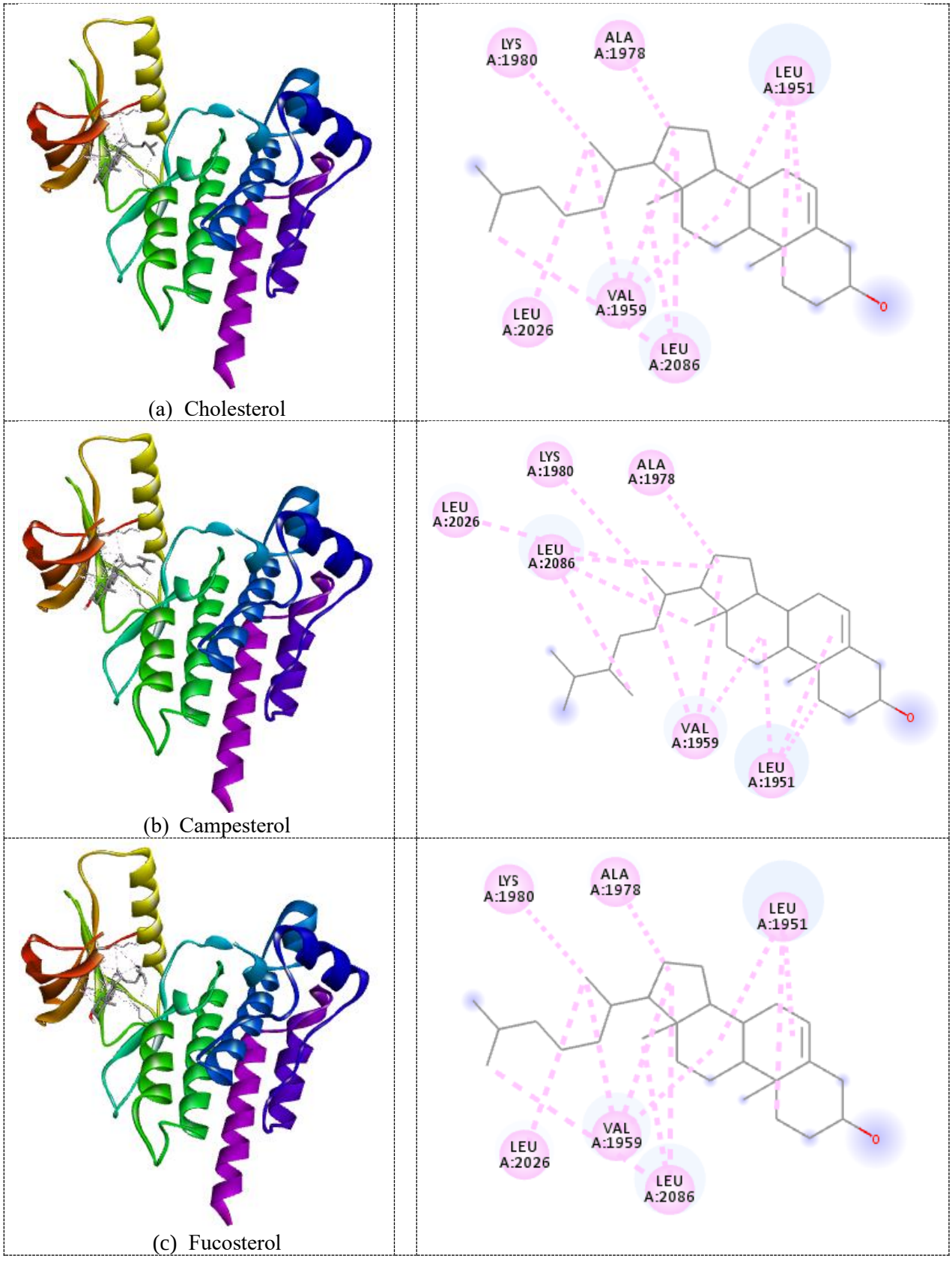


Figure 4. Interaction with Human ROS1 Kinase Receptor 3ZBF

3.2. Physicochemical Properties, Pharmacokinetics, Drug-Likeness, And Bioavailability of Selected Steroid Compounds

Drug-likeness and bioavailability were assessed based on six criteria: molecular size, hydrophobicity, polarity, solubility, degree of saturation, and flexibility (Table 3). Cholesterol fits well within the suitable physicochemical range for oral bioavailability with a molecular size of 386.65 g/mol (within the range of 150 - 500 g/mol), a polarity (TPSA) of 20.23 Å2 (within the range of 20 - 130 Å2), a degree of saturation (Fraction Csp3) of 0.93 (falling between 0.25 and 1.0), and shows flexibility with five rotatable bonds (not exceeding 9). However, it falls outside the acceptable range for lipophilicity and solubility, with XLOGP3 at 6.34 and log S (ESOL) at -7.40. Nevertheless, the drug-likeness analysis reveals only one violation of Lipinski's rule (MLOGP 6.34) and no violations of Veber's rule. The bioavailability score was 0.55.

Table 3. Physicochemical Properties, and Drug-Likeness, of Selected Steroid Compounds

Ligand	Lipinski's Rules*					Veber's rule**	
	MW (g/mol)	HBA	HBD	LogP	Molar Refractivity	RB	TPSA (Å^2)
Cholesterol	386.65	1	1	6.34	123.61	5	20.23
Campesterol	386.65	1	1	6.34	123.87	5	20.23
Fucosterol	470.77	1	1	6.62	132.75	5	20.23

* Lipinski's rule: MW ≤ 500g/mol; HBA ≤ 10; HBD ≤ 5, LogP ≤ 4.15 [24]
**Veber's rule: RB ≤ 9, TPSA 20 - 130 Å^2 [25]

Campesterol fits within the appropriate physicochemical parameters for oral bioavailability, with a molecular size of 386.65 g/mol (within the range of 150 - 500 g/mol), a polarity (TPSA) of 20.23 Å2 (within the range of 20 - 130 Å2), a degree of saturation (Fraction Csp3) of 0.93 (falling between 0.25 and 1.0), and exhibits flexibility with five rotatable bonds (not exceeding 9). However, it falls outside the acceptable range for lipophilicity and solubility, with XLOGP3 at 8.40 and log S (ESOL) at -7.20. Nevertheless, the drug-likeness analysis reveals only one violation of Lipinski's rule (MLOGP 6.34) and no violations of Veber's rule. The bioavailability score was 0.55.

Fucosterol also complies with the suitable physicochemical parameters for oral bioavailability, with a molecular size of 470.77 g/mol (within the range of 150 - 500 g/mol), a polarity (TPSA) of 20.23 Å2 (within the range of 20 - 130 Å2), a degree of saturation (Fraction Csp3) of 0.86 (between 0.25 and 1.0), and demonstrates flexibility with five rotatable bonds (not exceeding 9). However, it falls outside the acceptable range for lipophilicity and solubility, with XLOGP3 at 8.85 and log S (ESOL) at -7.64. Nevertheless, the drug-likeness analysis reveals only one violation of Lipinski's rule (MLOGP 6.62) and no violations of Veber's rule. The bioavailability score was 0.55.

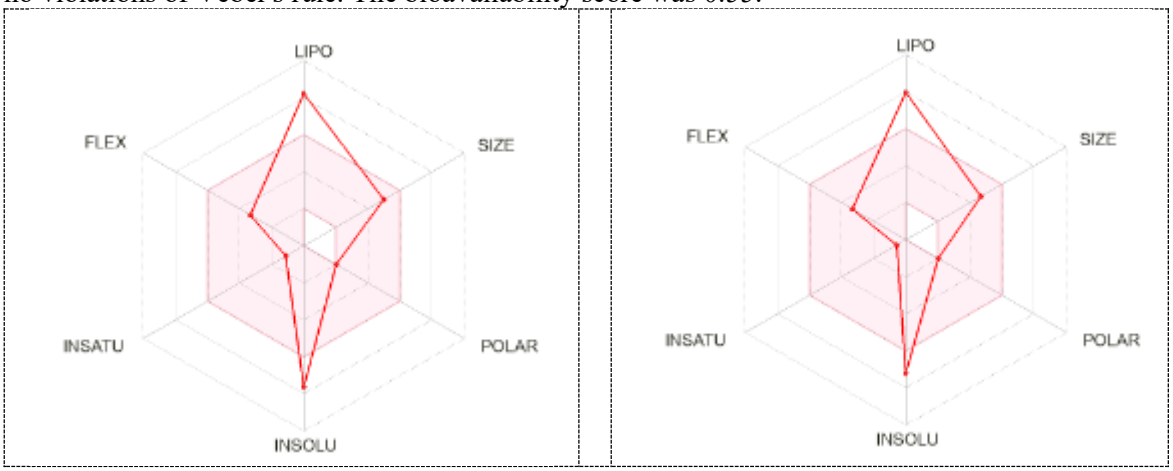


Figure 5. The bioavailability radar of Selected Steroid Compounds. a. Fucosterol, b. Campesterol

4. Conclusion

Stigmasterol, β-sitosterol, fucosterol, cholesterol, and campesterol exhibited binding affinities of -8.1, -8.2, -8.6, -8.4, and -8.5 kcal/mol, respectively, towards the 3ZBF human ROS1 kinase receptor. In contrast, crizotinib and ascorbic acid showed binding affinities of -8.4 kcal/mol and -4.7 kcal/mol. Fucosterol and campesterol demonstrated stronger binding affinities compared to crizotinib and ascorbic acid. Moreover, these compounds conformed to Lipinski's and Veber's rules and achieved a bioavailability score of 0.55, indicating that fucosterol and campesterol heve the potential as inhibitors of the human ROS1 kinase.

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