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IN SILICO MOLECULAR TARGETING AND ADMET PROFILING OF BETA-SITOSTEROL AS A MULTI-TARGET THERAPEUTIC CANDIDATE

Penargetan Molekuler Secara In Silico dan Profil ADMET Beta-Sitosterol sebagai Kandidat Terapeutik Multi-Target

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ABSTRACT
Beta-sitosterol is a naturally occurring phytosterol with potential biological activities, including anticancer effects. This study aimed to evaluate the pharmacokinetic characteristics and molecular targets of beta-sitosterol using in silico approaches involving ADMET analysis and target prediction. The ADMET results indicated that beta-sitosterol exhibited favorable membrane permeability and high absorption potential; however, it demonstrated poor aqueous solubility and high plasma protein binding, which may affect its bioavailability. Additionally, metabolic prediction suggested potential involvement of cytochrome P450 enzymes in beta-sitosterol biotransformation. Target prediction analysis revealed that beta-sitosterol may interact with multiple proteins involved in lipid metabolism and hormonal regulation. The highest predicted affinity was observed toward NPC1L1, a protein involved in cholesterol absorption, as well as nuclear receptors including LXR-α, ROR-γ, and androgen receptor, which regulate lipid metabolism and hormone signaling. Furthermore, beta-sitosterol was predicted to interact with HMG-CoA reductase and several cytochrome P450 isoforms involved in cholesterol biosynthesis and steroidogenesis. The findings suggest that beta-sitosterol possesses a multi-target profile associated with lipid metabolism and hormonal regulation pathways.

***Keywords**: Beta-sitosterol; In silico; SwissADME; SwissTargetPrediction; ADMET*

ABSTRAK
Beta-sitosterol merupakan fitosterol alami yang memiliki potensi aktivitas biologis, termasuk sebagai agen antikanker. Penelitian ini bertujuan untuk mengevaluasi karakteristik farmakokinetik dan target molekuler beta-sitosterol melalui pendekatan in silico menggunakan analisis ADMET dan target prediction. Hasil analisis ADMET menunjukkan bahwa beta-sitosterol memiliki permeabilitas membran yang baik dan potensi absorpsi yang tinggi, namun menunjukkan kelarutan air yang rendah serta ikatan protein plasma yang tinggi, yang dapat memengaruhi bioavailabilitasnya. Selain itu, prediksi metabolisme menunjukkan kemungkinan keterlibatan enzim sitokrom P450 dalam biotransformasi beta-sitosterol. Analisis target prediction menunjukkan bahwa beta-sitosterol berpotensi berinteraksi dengan berbagai protein yang terlibat dalam metabolisme lipid dan regulasi hormonal. Target dengan afinitas tertinggi meliputi NPC1L1 yang berperan dalam absorpsi kolesterol, serta reseptor nuklir seperti LXR-α, ROR-γ, dan androgen receptor yang berperan dalam regulasi metabolisme lipid dan sinyal

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hormon. Selain itu, beta-sitosterol juga diprediksi berinteraksi dengan enzim HMG-CoA reductase dan beberapa isoform sitokrom P450 yang terlibat dalam biosintesis kolesterol dan steroidogenesis. Hasil penelitian menunjukkan bahwa beta-sitosterol memiliki profil target multi-molekuler yang berkaitan dengan metabolisme lipid dan regulasi hormonal.

Kata kunci: *Beta-sitosterol; In silico; SwissADME; SwissTargetPrediction; ADMET*

INTRODUCTION

Beta-sitosterol has gained considerable attention because of its diverse pharmacological activities, including anti-inflammatory, antioxidant, immunomodulatory, and anticancer properties (Patel, J. *et al.,* 2023). Beta-sitosterol is a naturally occurring plant sterol present abundantly in various medicinal plants, and one promising botanical source is pumpkin seeds. Pumpkin seeds have long been used in traditional medicine for various health conditions, and modern studies have suggested their potential therapeutic roles, particularly due to their high content of valuable bioactive compounds including sterols, fatty acids, polyphenols, and micronutrients (Wang, R., *et al*., 2023). Beta-sitosterol has demonstrated potential in modulating pathways associated with cancer progression, apoptosis, and cell cycle regulation, suggesting its possible role in inhibiting carcinogenesis (Santos, L. J., *et al*., 2023).

Despite these potential advantages, the development of beta-sitosterol as a therapeutic agent requires a comprehensive understanding of its molecular interactions with cancer-associated targets (Ahmed, M., *et al*., 2024). Molecular targeting studies play a critical role in identifying the precise mechanisms by which beta-sitosterol exerts anticancer effects, such as binding affinity to androgen receptors, modulation of inflammatory mediators, or inhibition of enzymes that regulate cell proliferation (Menon, A., *et al*., 2024). Computational and in silico approaches have significantly advanced the field of drug discovery by enabling efficient screening of phytochemicals against multiple biological targets (El-Sayed, A. E. *et al*., 2023). Molecular docking, molecular dynamics simulation, and binding free energy calculations can provide detailed insights into the molecular basis of ligand–target interactions (Sari, R., *et al*., 2023). These approaches are essential for predicting whether beta-sitosterol could act selectively and effectively on specific proteins implicated in prostate cancer progression (Lee, H., *et al*., 2024). The ability to analyze structural compatibility, interaction stability, and conformational changes between beta-sitosterol and cancer-related proteins helps strengthen the rationale for developing this compound as a therapeutic agent (Ibrahim, M., *et al.,* 2024).

In addition to assessing molecular targeting, evaluation of the ADMET profile—Absorption, Distribution, Metabolism, Excretion, and Toxicity is fundamental in determining the feasibility of beta-sitosterol as a drug candidate (Osei, J., *et al*., 2024). ADMET properties play a decisive role in defining a compound's pharmacokinetic behavior, systemic availability, and safety profile in humans (Kumar, K., 2024). Poor absorption, rapid metabolism, or high toxicity can significantly hinder the development of promising anticancer agents even when they exhibit potent biological activity in vitro (Rahman, A., *et al.,* 2023). Therefore, computational ADMET prediction tools have become indispensable for identifying potential pharmacokinetic challenges and optimizing drug-like characteristics early in the research process (Mohan, S., & Jain, A., 2024). For natural compounds such as beta-sitosterol, whose solubility and bioavailability are often limited, ADMET profiling can reveal critical information on modifications or delivery strategies that may be required to enhance therapeutic potential (Ozkan, H. *et al*, 2024). Moreover, examining toxicity predictions ensures that the compound does not pose harmful effects on vital organs, which is a common barrier in anticancer drug development (Choudhary, A., & Verma, R., 2023).

Pumpkin seeds represent a natural source of beta-sitosterol, supporting their potential for further pharmacological investigation (Silva, A., *et al*., 2023). Traditional uses of pumpkin seeds in treating urinary and reproductive disorders imply possible synergy with therapeutic strategies for prostate diseases, including benign prostatic hyperplasia and prostate cancer (Zhang, L. *et al*., 2023). The growing interest in plant sterols as anticancer candidates is supported by mounting evidence that such compounds can modulate lipid homeostasis, hormone signaling, and cellular stress responses (Gupta, H., *et al*., 2023). These physiological pathways are highly relevant to prostate cancer development, which is influenced by androgen regulation, oxidative stress, chronic inflammation, and metabolic dysregulation (Mathew, D., *et al*., 2024). the evaluation of beta-sitosterol through molecular targeting and ADMET profiling represents a crucial step in advancing natural product-based therapeutic development (Patel, R., *et al*., 2024).

Modern computational methodologies offer a practical and cost-effective means to accelerate early-stage drug discovery, particularly for natural compounds that require extensive characterization before advancing to laboratory or clinical evaluation. In silico studies allow researchers to predict binding interactions, identify potential molecular targets, and assess drug-likeness parameters without the need for large quantities of purified compounds (Ndlovu, T.,*et al*., 2023). These methods have been widely adopted in pharmaceutical research because they can rapidly generate reliable predictions and guide subsequent experimental validation. For beta-sitosterol, such predictions can help clarify its anticancer mechanism and determine whether its structural characteristics are compatible with key prostate cancer molecular targets. Incorporating ADMET modeling further refines the prediction of clinical feasibility by analyzing how the compound may behave within the human body (Ahmed, F., *et al*., 2023).

Natural products have historically contributed to the discovery of numerous therapeutic agents, and phytosterols represent a promising class of bioactive compounds with potential pharmacological benefits. Beta-sitosterol has gained attention due to its diverse biological activities and potential as a multi-target therapeutic candidate. Molecular targeting approaches can help identify potential protein interactions, while ADMET profiling provides insight into pharmacokinetic and toxicity properties relevant to drug development. Therefore, this study aims to evaluate the molecular targets and ADMET profile of beta-sitosterol to assess its potential as a multi-target therapeutic candidate.

METHODOLOGY

This study employed an in silico computational approach to evaluate the potential of beta-sitosterol as a multi-target therapeutic candidate.

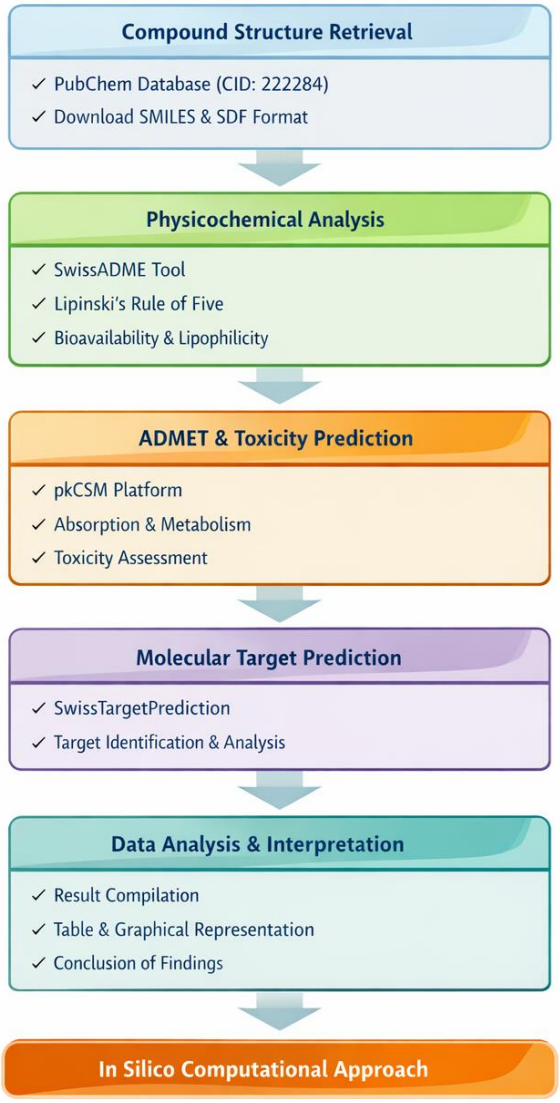

Figure 1. Flow Chart

The research consisted of several stages, including compound structure retrieval, physicochemical property analysis, pharmacokinetic prediction, toxicity assessment, and molecular target identification. All procedures were conducted using widely accepted web-based computational platforms commonly applied in pharmaceutical research.

1. Compound Structure Retrieval

The chemical structure of beta-sitosterol was obtained from the PubChem database. PubChem provides comprehensive information regarding molecular structures, chemical properties, and biological data for various chemical compounds. The compound was searched using the keyword "beta-sitosterol" in the search field. Beta-sitosterol (CID: 222284) was retrieved from the PubChem database (accessed December 15, 2026). After identification, the chemical structure was downloaded in canonical SMILES and SDF formats. These structural data were subsequently used as input for further computational analysis. PubChem was selected due to its reliability, accessibility, and validation in numerous in silico studies.

2. Physicochemical Properties and Drug-Likeness Analysis

Physicochemical properties and drug-likeness evaluation of beta-sitosterol were performed using the SwissADME web server. SwissADME was utilized to assess molecular characteristics associated with drug development suitability. The analyzed parameters included molecular weight, hydrogen bond donors, hydrogen bond acceptors, lipophilicity, and topological polar surface area. In addition, Lipinski's rule of five was evaluated to determine oral drug-likeness. Additional parameters analyzed included bioavailability score, number of rotatable bonds, and solubility. Gastrointestinal absorption prediction was also performed to assess oral bioavailability potential. Furthermore, blood–brain barrier permeability was evaluated to determine central nervous system distribution potential.

SwissADME also provides bioavailability radar visualization, which helps in understanding pharmacokinetic characteristics. Lipophilicity predictions were calculated using multiple models, including iLOGP, XLOGP, and WLOGP. These results were compared to obtain a more comprehensive assessment of lipophilicity.

3. Pharmacokinetics and Toxicity Prediction

Pharmacokinetic and toxicity prediction were conducted using the pkCSM web server. pkCSM was used to evaluate ADMET properties, including absorption, distribution, metabolism, excretion, and toxicity. Absorption parameters included intestinal absorption and skin permeability. Distribution analysis included volume of distribution and blood–brain barrier permeability.

Metabolism prediction included interactions with cytochrome P450 enzymes. These parameters are essential for understanding metabolic pathways. Excretion analysis included total clearance and renal clearance predictions. Toxicity assessment included hepatotoxicity, mutagenicity, and carcinogenicity predictions. Additional toxicity parameters included Ames toxicity prediction and LD50 estimation. These parameters are important for evaluating compound safety. The pkCSM platform provides a comprehensive evaluation of pharmacokinetic and toxicity characteristics for drug development.

4. Molecular Target Prediction

Molecular target prediction was performed using the SwissTargetPrediction web server. The canonical SMILES of beta-sitosterol was used as input. SwissTargetPrediction identifies potential protein targets based on chemical similarity with known ligands.

Predicted targets were analyzed based on probability values. Targets with higher probability scores were selected as potential targets. Protein classification was performed to categorize predicted targets into enzyme, receptor, ion channel, or transporter groups. Further analysis was conducted to determine biological functions and pharmacological relevance. The predicted targets were compiled into tables for further interpretation.

5. Data Analysis

Data obtained from each platform were analyzed descriptively. Physicochemical data were evaluated based on Lipinski's rule of five. ADMET data were analyzed based on standard pharmacokinetic

parameters. Molecular target prediction data were analyzed based on probability scores. Results were presented in tables and graphical representations. Interpretation was performed to determine the potential of beta-sitosterol as a multi-target therapeutic candidate.

6. Research Workflow

The research workflow consisted of several stages. The first stage involved compound structure retrieval. The second stage involved physicochemical analysis. The third stage involved ADMET prediction. The fourth stage involved molecular target prediction. The final stage involved data analysis and interpretation. Each stage was conducted sequentially to ensure systematic workflow and data reliability.

7. Methodological Validation

The methodology used in this study has been widely applied in computational drug discovery research. The selected platforms provide validated predictive models. The use of multiple platforms improves accuracy and reliability. This in silico approach also offers advantages in terms of efficiency, cost-effectiveness, and time-saving. Therefore, this computational approach is suitable for preliminary drug discovery and development studies.

RESULTS AND DISCUSSION

A comprehensive understanding of the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) characteristics of a bioactive compound is a fundamental component of early-stage drug discovery and development. ADMET profiling provides essential insights into how a molecule behaves within a biological system, including its pharmacokinetic fate, interaction with metabolic enzymes, potential toxicity risks, and the extent to which it can achieve therapeutically relevant concentrations at target tissues. In the context of natural product–based drug development, in silico ADMET prediction has become a powerful and efficient tool that enables rapid evaluation of multiple pharmacokinetic parameters, reduces experimental costs, and helps prioritize promising candidates before moving into in vitro and in vivo validation. Despite its well-established pharmacological activities, the compound's pharmacokinetic behavior poses several uncertainties, particularly regarding its solubility, permeability, metabolic pathways, and safety profile. Therefore, computational ADMET modeling is crucial to elucidate beta-sitosterol's drug-likeness, predict potential liabilities, and assess its suitability as a candidate therapeutic agent.

Table 1. Profil ADMET Beta-Sitosterol

Category	Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	Water solubility	-7.565	log mol/L
Absorption	Caco2 permeability	Caco2 permeability	1.331	log Papp (10^{-6} cm/s)
Absorption	Intestinal absorption (human)	Intestinal absorption	92.986	% Absorbed
Absorption	Skin permeability	Skin permeability	-2.802	log Kp
Absorption	P-glycoprotein substrate	P-gp substrate	Yes	Yes/No
Absorption	P-glycoprotein I inhibitor	P-gp I inhibitor	Yes	Yes/No
Absorption	P-glycoprotein II inhibitor	P-gp II inhibitor	Yes	Yes/No
Distribution	VDss (human)	VDss	1.161	log L/kg
Distribution	Fraction unbound (human)	Fraction unbound	0	Fu
Distribution	BBB permeability	BBB permeability	0.504	log BB
Distribution	CNS permeability	CNS permeability	-0.983	log PS
Metabolism	CYP2D6 substrate	CYP2D6 substrate	No	Yes/No
Metabolism	CYP3A4 substrate	CYP3A4 substrate	Yes	Yes/No
Metabolism	CYP1A2 inhibitor	CYP1A2 inhibitor	No	Yes/No
Metabolism	CYP2C19 inhibitor	CYP2C19 inhibitor	No	Yes/No
Metabolism	CYP2C9 inhibitor	CYP2C9 inhibitor	No	Yes/No

Category	Property	Model Name	Predicted Value	Unit
Metabolism	CYP2D6 inhibitor	CYP2D6 inhibitor	No	Yes/No
Metabolism	CYP3A4 inhibitor	CYP3A4 inhibitor	No	Yes/No
Excretion	Total Clearance	Total clearance	0.591	log mL/min/kg
Excretion	Renal OCT2 substrate	Renal OCT2 substrate	No	Yes/No
Toxicity	AMES toxicity	AMES toxicity	No	Yes/No
Toxicity	Max. tolerated dose (human)	MTD	0.613	log mg/kg/day
Toxicity	hERG I inhibitor	hERG I inhibitor	No	Yes/No
Toxicity	hERG II inhibitor	hERG II inhibitor	Yes	Yes/No
Toxicity	Oral Rat Acute Toxicity (LD50)	LD50	2.063	mol/kg
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	LOAEL	0.921	log mg/kg_bw/day
Toxicity	Hepatotoxicity	Hepatotoxicity	No	Yes/No
Toxicity	Skin Sensitisation	Skin sensitisation	No	Yes/No
Toxicity	T. *Pyriformis* toxicity	T. *Pyriformis* toxicity	0.858	log µg/L
Toxicity	Minnow toxicity	Minnow toxicity	-2.939	log mM

The absorption profile of beta-sitosterol, as represented in the table, highlights several important pharmaceutical challenges and opportunities. The predicted water solubility value (-7.565 log mol/L) may indicate that beta-sitosterol is poorly soluble in aqueous media, a characteristic commonly observed in sterol scaffolds that may potentially limit oral dissolution and bioavailability. Despite this poor intrinsic solubility, the compound shows a favorable Caco-2 permeability score (1.331 log Papp in 10^{-6} cm/s) and a very high predicted human intestinal absorption (≈92.99% absorbed), which suggests that once dissolved or presented in an absorbable form, beta-sitosterol can permeate enterocyte monolayers effectively. The skin permeability (log Kp = -2.802) is consistent with low dermal absorption—a negative for transdermal delivery strategies but not surprising for a bulky lipophilic sterol. Crucially, the predictions mark beta-sitosterol as a P-glycoprotein substrate and also may indicate inhibitory activity against P-gp I and II. This dual profile implies that intestinal absorption and tissue distribution may be modulated by efflux transporters: P-gp could limit effective systemic exposure by pumping the compound back into the intestinal lumen or out of sensitive tissues, while P-gp inhibition could either increase intracellular exposure of co-administered drugs or alter the compound's own pharmacokinetics when transporter expression/activity changes. Taken together, the absorption data point toward formulation- or delivery-focused approaches (solubility enhancement, lipid-based carriers, cyclodextrin complexation, nanoformulations, or prodrugs) to convert the favorable permeability potential into reliable systemic exposure, while also flagging transporter-mediated interactions as an important consideration for both efficacy and drug–drug interaction risk.

The distribution parameters paint a picture of a strongly tissue-distributing, highly protein-associated molecule. A VDss value of 1.161 (log L/kg) corresponds to a volumetric distribution substantially greater than plasma volume, which may suggest extensive partitioning into tissues and possibly adipose compartments consistent with high lipophilicity. The fraction unbound in human plasma is predicted as 0 (Fu ≈ 0), suggesting near-complete plasma protein binding in silico; in practice this will mean a very low free (pharmacologically active) fraction in plasma and a dependence of pharmacodynamic effects on tissue reservoirs and slow release. Blood–brain barrier permeation (log BB = 0.504) being positive suggests likely central nervous system (CNS) penetration, and the CNS permeability metric (log PS = -0.983)—moderately negative—may indicate limited passive permeation rate relative to highly permeable CNS drugs.

The combination of high VDss and predicted BBB permeability may suggest

potential tissue distribution of beta-sitosterol; however, these findings remain theoretical and require experimental validation. Lipophilic phytosterols such as beta-sitosterol have been reported to exhibit tissue distribution characteristics due to their structural similarity to cholesterol and membrane affinity, which may facilitate accumulation in peripheral tissues. Previous pharmacokinetic studies have demonstrated that beta-sitosterol exhibits a relatively large apparent volume of distribution and prolonged residence time, supporting the possibility of tissue distribution beyond plasma compartments (Sayeed *et al*., 2016). Additionally, experimental studies have reported that plant sterols, including sitosterol, can be detected in brain tissue under certain physiological conditions, suggesting the potential for central nervous system exposure, although the extent of penetration remains limited and condition-dependent (Saeed *et al*., 2015).

Because the free fraction is predicted to be low, pharmacological effects may be influenced by slow equilibrium between tissue stores and plasma concentrations. However, this interpretation should be considered cautiously, as pharmacokinetic behavior derived from in silico predictions may not fully reflect in vivo conditions. Therefore, further experimental pharmacokinetic studies are required to confirm tissue distribution, CNS exposure, and dose–response relationships of beta-sitosterol.

Metabolic predictions highlight a principal role for CYP3A4 in the biotransformation of beta-sitosterol. Based on the prediction results, beta-sitosterol may act as a substrate of CYP3A4 but not CYP2D6, and it is not predicted to significantly inhibit major CYP isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4). This finding is consistent with previous studies reporting that lipophilic phytosterols are primarily metabolized by CYP3A4-mediated pathways, reflecting the enzyme's dominant role in the metabolism of sterol-like compounds (Wang *et al*., 2023; Miszczuk *et al*., 2024). Since CYP3A4 is responsible for metabolizing approximately 30–50% of clinically used drugs, compounds primarily metabolized by CYP3A4 are susceptible to pharmacokinetic variability and drug–drug interactions (Zhou *et al.,* 2023).

Being primarily metabolized by CYP3A4 has two immediate implications. First, the compound's clearance and systemic exposure may be sensitive to coadministration with CYP3A4 inducers or inhibitors. For example, grapefruit constituents, rifampicin, and certain antifungal agents are known to modulate CYP3A4 activity and alter drug metabolism, potentially affecting pharmacokinetic behavior (Hodges & Minich, 2023; Yu *et al*., 2022). Second, interindividual variability in CYP3A4 expression may contribute to variable pharmacokinetics among patient populations.

The lack of predicted major CYP inhibition reduces the likelihood that beta-sitosterol acts as a perpetrator of pharmacokinetic drug–drug interactions via CYP inhibition. However, because it is predicted to be a substrate, it remains vulnerable to being affected by interactions. Further in vitro studies using human liver microsomes and recombinant CYP isoforms, along with metabolite structure characterization, are recommended to validate in silico predictions. Such approaches have been widely employed in recent studies to identify metabolic pathways, enzyme involvement, and potential active or toxic metabolites of bioactive compounds (Chen *et al*., 2023; Lee *et al*., 2024; Zhou *et al*., 2022).

Excretion and clearance predictions are moderate and consistent with a compound that distributes broadly but is not rapidly eliminated. A total clearance of 0.591 (log mL/min/kg) corresponds to a modest systemic clearance rate; this is compatible with the high VDss and high protein binding, which together often produce prolonged terminal half-lives. The absence of renal OCT2 substrate activity suggests that active tubular secretion via OCT2 is not a primary elimination route, and elimination may instead rely on hepatic metabolism and biliary excretion—again consistent with the CYP3A4 substrate prediction and with the sterol chemical class. For development, slower clearance can be advantageous for sustaining target exposures but raises concerns about accumulation, especially with chronic dosing, and mandates appropriate dosing

interval design and monitoring in safety studies.

The toxicity predictions offer a mixed but actionable safety signal. The model predicts a negative Ames toxicity result, implying a low likelihood of primary mutagenicity in standard bacterial assays, which is favorable for genotoxicity risk. Hepatotoxicity was predicted as negative and skin sensitization as negative, providing initial reassurance for hepatic and dermatologic safety in silico. However, two aspects require caution: (1) the compound is predicted to inhibit hERG II (human Ether-à-go-go-Related Gene class II), and while hERG I is negative, any hERG inhibition raises a red flag for possible cardiotoxicity and QT-interval prolongation liability; in vitro hERG channel assays and in vivo cardiovascular safety studies will therefore be essential. (2) The predicted maximum tolerated dose (MTD, log mg/kg/day = 0.613) and LOAEL (log mg/kg_bw/day = 0.921) suggest finite margins between efficacious and adverse exposures and emphasize the need to determine an empirical therapeutic index. The predicted oral rat acute toxicity (LD50) and aquatic toxicity metrics (T. pyriformis and minnow) are relevant for safety profiling and environmental impact but must be interpreted carefully because the units (especially LD50 in mol/kg) and the translation from in silico to in vivo are nontrivial.

Several practical consequences and development recommendations follow from these in silico insights. First, the very low aqueous solubility must be addressed through formulation science before any meaningful oral pharmacokinetic or efficacy studies are attempted; lipid-based formulations, self-emulsifying drug delivery systems, nanoparticle encapsulation, or cyclodextrin inclusion complexes are typical strategies for sterol compounds. Second, because beta-sitosterol is a P-gp substrate and P-gp inhibitor, transporter assays (Caco-2 bidirectional assays, MDCK-MDR1 cells) and in vivo studies should be conducted to quantify efflux ratios and to assess transporter-mediated drug interactions. Third, metabolic profiling focused on CYP3A4—identifying primary metabolites, clearance routes, and possible reactive intermediates—will clarify both efficacy and safety questions and help predict DDI risk. Fourth, cardiotoxicity risk mitigation requires prioritized in vitro hERG screening, followed by telemetry studies in relevant animal models if flags persist. Fifth, the predicted high tissue distribution but low free fraction suggests dose selection should be guided by free-drug concentrations at target sites and by modeling approaches (PBPK, physiologically based pharmacokinetic models) to anticipate accumulation and to design safe human dosing regimens.

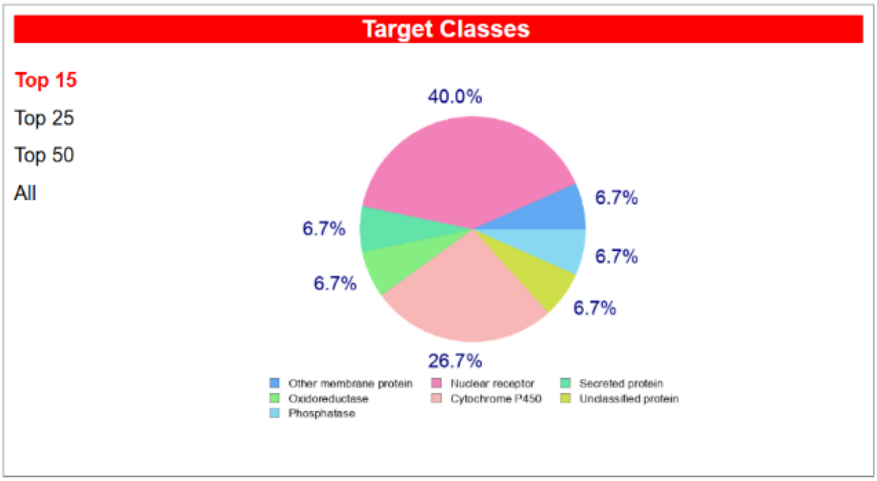

Figure 2. Target Classes

The target class distribution illustrated in the figure provides an important overview of the predicted molecular interactions of beta-sitosterol and offers insight into the biochemical pathways through which this phytosterol may exert therapeutic activity, particularly in prostate cancer. The pie chart demonstrates that nuclear receptors

constitute the largest class of predicted targets at 40%, which may indicate a strong likelihood that beta-sitosterol interacts with transcriptional regulators involved in hormone signaling, lipid metabolism, and cellular homeostasis. This finding is particularly noteworthy because nuclear receptors, including androgen receptors, peroxisome proliferator-activated receptors (PPARs), and estrogen receptors, regulate gene expression in response to lipid-based ligands. Beta-sitosterol, as a plant-derived sterol, possesses structural similarities to endogenous steroid hormones, which may enable it to modulate receptor-mediated signaling pathways. Such interactions are highly relevant, particularly in hormone-dependent conditions, where modulation of nuclear receptor activity may influence cell proliferation, apoptosis, and metabolic regulation.

Cytochrome P450 enzymes represent the second largest class of predicted targets at 26.7%, suggesting that beta-sitosterol may also interact with metabolic enzymes responsible for xenobiotic transformation, steroid metabolism, and cellular detoxification. These interactions may influence the pharmacokinetics of beta-sitosterol and alter metabolic pathways associated with cellular homeostasis. In particular, members of the CYP3A subfamily are known to play significant roles in steroid metabolism, oxidative biotransformation, and drug clearance. Therefore, predicted interactions with cytochrome P450 enzymes may contribute to modulation of metabolic processes and pharmacokinetic behavior, although further experimental validation is required.

Other membrane proteins represent 6.7% of the predicted targets, suggesting that beta-sitosterol may interact with membrane-bound receptors or transporters, potentially modulating signal transduction, cell communication, or membrane stability. Another 6.7% is represented by oxidoreductases, which are enzymes involved in redox balance, oxidative stress regulation, and metabolic reactions. This interaction aligns with evidence that beta-sitosterol possesses antioxidant properties that may contribute to the reduction of oxidative damage within cancer cells. The phosphatase class, also comprising 6.7%, indicates potential modulation of phosphorylation–dephosphorylation pathways, which regulate cell cycle progression, tumor suppressor activity, and apoptosis signaling.

Finally, 6.7% of targets fall into the unclassified protein group, reflecting proteins that do not fit into conventional classification categories but may still play relevant biological roles. Collectively, the distribution of target classes suggests that beta-sitosterol exerts a multifaceted mechanism of action through interactions with metabolic enzymes, nuclear receptors, membrane proteins, redox regulators, and signaling proteins.

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*
Niemann Pick C1 like protein 1	NPC1L1	Q9UHC9	CHEMBL2027	Other membrane protein	
LXR-alpha	NR1H3	Q13133	CHEMBL2808	Nuclear receptor	
Nuclear receptor ROR-gamma	RORC	P51449	CHEMBL1741186	Nuclear receptor	
Testis-specific androgen-binding protein	SHBG	P04278	CHEMBL3305	Secreted protein	
HMG-CoA reductase	HMGCR	P04035	CHEMBL402	Oxidoreductase	
Cytochrome P450 17A1	CYP17A1	P05093	CHEMBL3522	Cytochrome P450	
Sterol regulatory element-binding protein 2	SREBF2	Q12772	CHEMBL1795166	Unclassified protein	
Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978	Cytochrome P450	
Androgen Receptor	AR	P10275	CHEMBL1871	Nuclear receptor	
Cytochrome P450 51 *(by homology)*	CYP51A1	Q16850	CHEMBL3849	Cytochrome P450	
Nuclear receptor ROR-alpha	RORA	P35398	CHEMBL5868	Nuclear receptor	
Estrogen receptor alpha	ESR1	P03372	CHEMBL206	Nuclear receptor	
Estrogen receptor beta	ESR2	Q92731	CHEMBL242	Nuclear receptor	
Protein-tyrosine phosphatase 1B	PTPN1	P18031	CHEMBL335	Phosphatase	

Figure 3. Probability Target

The target-prediction analysis of beta-sitosterol suggests that this compound may interact with multiple protein classes involved in lipid metabolism, hormonal regulation, and cancer-related pathways, including those relevant to prostate cancer. Previous studies have demonstrated that beta-sitosterol exhibits biological activities such as modulation of lipid metabolism, anti-inflammatory effects, and anticancer potential through regulation of signaling pathways associated with cell proliferation and apoptosis. Additionally, phytosterols structurally resemble cholesterol and steroid hormones, which may contribute to their interaction with proteins involved in hormonal regulation and carcinogenic processes. These findings support the biological plausibility of the predicted targets obtained from in silico analysis, although further experimental validation is required. The target with the highest predicted binding probability is Niemann-Pick C1-like protein 1 (NPC1L1), an “other membrane protein” responsible for cholesterol absorption. The affinity of beta-sitosterol for NPC1L1 supports its competitive mechanism in inhibiting cholesterol uptake, which may indirectly reduce cholesterol availability required for the proliferation of prostate cancer cells, given their strong dependence on lipid metabolism (Shen *et al*., 2024). In addition, beta-sitosterol is predicted to interact with LXR-alpha (NR1H3) and the nuclear receptor ROR-gamma (RORC), both belonging to the nuclear receptor class—key transcriptional regulators of inflammation, lipid metabolism, and cell proliferation. These interactions are significant because the transcriptional pathways governed by these receptors have been linked to prostate cancer development through modulation of androgen signaling, chronic inflammation, and lipid homeostasis (Abdel-Rasol & El-Sayed, 2025).

Predicted targets of beta-sitosterol include sex hormone-binding globulin (SHBG), a secreted protein that plays a crucial role in binding and transporting androgens and estrogens in circulation. The predicted affinity of beta-sitosterol for SHBG suggests its potential to modulate free androgen levels, thereby influencing androgen-dependent signaling pathways that are central to the progression of prostate cancer. Additionally, beta-sitosterol is predicted to interact with HMG-CoA reductase (HMGCR), a key enzyme involved in cholesterol biosynthesis. Interaction with HMGCR may contribute to reduced cellular cholesterol levels, which could subsequently suppress the proliferation of prostate cancer cells, as these cells rely heavily on cholesterol for membrane formation and lipid-signaling pathways. This observation is consistent with previous studies indicating that cholesterol-lowering mechanisms may contribute to anticancer effects, particularly in hormone-dependent cancers such as prostate cancer (Durrani, A. K., *et al*., 2024).

Predicted targets of beta-sitosterol include several members of the cytochrome P450 (CYP) family, such as CYP17A1, CYP19A1, and CYP51A1. These enzymes play essential roles in steroid hormone metabolism and xenobiotic detoxification. For instance, CYP17A1 is critically involved in androgen biosynthesis, suggesting that interaction between beta-sitosterol and this enzyme may influence hormone production associated with prostate cancer progression (Cabeza, M., *et al*., 2025).

These predicted interactions indicate that beta-sitosterol may modulate steroidogenesis pathways, which represent clinically relevant therapeutic targets in androgen-dependent prostate cancer. Such findings further support the potential role of beta-sitosterol in influencing hormone-related cancer pathways, although experimental validation is necessary to confirm these predicted interactions (Saini, H., *et al*., 2024).

Critical target is the androgen receptor (AR), a nuclear receptor that mediates cellular responses to androgens. The presence of AR in the predicted target list indicates that beta-sitosterol may possess the ability to interact with this receptor, which is central to the proliferation of prostate cancer cells. Such interaction implies a potential anti-androgenic mechanism that could inhibit AR-mediated transcriptional activity, thereby slowing cancer cell growth. In addition to AR, the detection of estrogen receptors ESR1 and ESR2 suggests that beta-sitosterol may also modulate estrogenic activity, an important aspect of hormonal balance and its relevance to prostate cancer biology (Chen, J., *et al*., 2024).

The analysis predicts interaction with protein-tyrosine phosphatase 1B (PTPN1), a phosphatase involved in insulin resistance and cellular signaling regulation. The potential binding of beta-sitosterol to PTPN1 enriches the understanding of its metabolic modulatory effects, which may contribute to anticancer properties through the regulation of oxidative stress and proliferative pathways.

CONCLUSION

This study evaluated the pharmacokinetic properties and potential molecular targets of beta-sitosterol using in silico ADMET prediction and target prediction approaches. The results suggest that beta-sitosterol possesses favorable permeability and a broad range of predicted targets associated with lipid metabolism and hormonal regulation. However, its poor aqueous solubility and high plasma protein binding may present potential pharmacokinetic limitations. The predicted interactions with proteins involved in cholesterol metabolism and androgen signaling indicate that beta-sitosterol may have potential relevance in prostate cancer–related pathways. Nevertheless, these findings are based solely on computational predictions and therefore require further validation through molecular docking studies, as well as in vitro and in vivo experiments.

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