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Evaluation of GSTP1 Inhibition Potentials and Pharmacokinetic Properties of Stigmasterol, Sesamin, and Pinosylvin

Stigmasterol, Sesamin ve Pinosilvin'in GSTP1 İnhibisyon Potansiyellerinin ve Farmakokinetik Özelliklerinin Değerlendirilmesi

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ABSTRACT

Objective: Glutathione S-transferase P1 (GSTP1), an important target affecting drug resistance in cancer treatment, is a critical issue due to its role in detoxifying and regulating reactive oxygen species. This study evaluated the inhibitory potentials of natural compounds (bakuchiol, sesamin, hydroxytyrosol, stigmasterol, and pinosylvin) against Glutathione S-transferase P1 and their absorption, metabolism, distribution, and elimination (ADME) profiles.

Material and Method: The inhibitory activities of these compounds were compared with those of the reference inhibitor, etacrynic acid, using molecular docking simulations and absorption, metabolism, distribution, and elimination profiling.

Results: Docking simulations showed that stigmasterol (-9.2 kcal/mol) and sesamin (-8.2 kcal/mol) exhibited the most potent binding affinities, followed by pinosylvin (-7.1 kcal/mol), surpassing etacrynic acid (-6.7 kcal/mol) in inhibition potential. Although the absorption, metabolism, distribution, and elimination analysis indicated risks related to solubility and enzyme interactions, it highlighted favorable pharmacokinetic properties for sesamin and pinosylvin.

Conclusion: This study emphasizes the potential of plant-derived compounds by targeting Glutathione S-transferase P1-mediated drug resistance. Such approaches may enable the development of new and effective strategies in cancer treatment.

Keywords: ADME profiling, cancer therapy, drug resistance, GSTP1, molecular docking, phytochemicals.

ÖZET

Amaç: Kanser tedavisinde ilaç direncini etkileyen önemli bir hedef olan Glutatyon S-transferaz P1 (GSTP1), detoksifikasyon ve reaktif oksijen türlerinin regülasyonundaki rolü nedeniyle kritik bir konudur. Bu çalışmada, doğal bileşiklerin (bakuchiol, sesamin, hidroksitirosol, stigmasterol ve pinosilvin) GSTP1'e karşı inhibitör potansiyelleri ve ADME (Absorpsiyon, metabolizma, dağılım, eliminasyon) profilleri değerlendirilmiştir.

Gereç ve Yöntem: Moleküler kenetleme simülasyonları ve absorpsiyon, metabolizma, dağılım, eliminasyon profillemesi kullanılarak, bu bileşiklerin inhibitör etkinlikleri referans inhibitör olan etakrinik asit ile karşılaştırılmıştır.

Bulgular: Kenetleme simülasyonları, stigmasterol (-9,2 kcal/mol) ve sesaminin (-8,2 kcal/mol) en güçlü bağlanma afinitelerini sergilediğini, ardından pinosilvin'in (-7,1 kcal/mol) inhibisyon potansiyelinde etakrinik asidi (-6,7 kcal/mol) geride bıraktığını gösterdi. Absorpsiyon, metabolizma, dağılım ve eliminasyon analizi, çözünürlük ve enzim etkileşimi riskleri belirtmiş olsa da sesamin ve pinosylvin için olumlu farmakokinetik özellikleri vurguladı.

Sonuç: Bu çalışma, Glutatyon S-transferaz P1 aracılı ilaç direncini hedef alarak, bitki kaynaklı bileşiklerin potansiyelini vurgulamaktadır. Bu tür yaklaşımlar, kanser tedavisinde yeni ve etkili stratejiler geliştirilmesine olanak sağlayabilir.

Anahtar Sözcükler: ADME profillemesi, fitokimyasallar, GSTP1, ilaç direnci, kanser tedavisi, moleküler yerleştirme.

Introduction

Cancer remains a leading cause of death globally, driving the need for innovative therapeutic approaches to overcome its challenges, particularly drug resistance. The glutathione detoxification pathway plays a significant role among the cellular defense mechanisms implicated in drug resistance. A central enzyme in this pathway, Glutathione S-transferase P1 (GSTP1), is known for its ability to neutralize reactive oxygen species (ROS) and detoxify harmful agents, including many chemotherapeutic drugs. GSTP1 is known to be overexpressed in a variety of malignancies, including breast, lung, and colorectal. This overexpression plays a key role in promoting tumor survival and resistance to therapies (1,2). As a result, GSTP1 has emerged as an important molecular target in cancer treatment, aiming to overcome resistance mechanisms and improve therapeutic outcomes.

Ethacrynic acid, initially developed as a diuretic, was one of the first compounds identified to inhibit GSTP1. It works by binding covalently to the enzyme's active site, effectively blocking its detoxification function (3). Despite its potential, its clinical use is restricted by unwanted side effects and less-than-ideal pharmacokinetic characteristics. These limitations highlight the need for alternative GSTP1 inhibitors that are not only effective but also associated with fewer adverse reactions. In this regard, natural compounds have gained attention as a promising option due to their diverse structures, broad biological activities, and generally favorable safety profiles.

A range of natural compounds, such as bakuchiol, sesamin, hydroxytyrosol, stigmasterol, and pinosylvin, have demonstrated considerable therapeutic potential. Bakuchiol, a meroterpene phenol obtained from Psoralea corylifolia, has been extensively researched for its antioxidant, anti-inflammatory, and anticancer effects. Notably, it has shown the ability to regulate oxidative stress and trigger apoptosis in cancer cells (4,5). Similarly, sesamin, a lignan derived from sesame seeds (Sesamum indicum), has shown significant anticancer properties, mainly by reducing ROS production and blocking inflammatory pathways (6,7). Hydroxytyrosol, a significant phenolic compound in olive oil, has attracted interest for its ability to protect cells from oxidative stress and promote apoptosis in cancer models (8).

Stigmasterol, a phytosterol found in several plant oils, has demonstrated anticancer effects by modulating cellular cholesterol metabolism and inhibiting the proliferation and migration of tumor cells (9,10). Pinosylvin, a stilbenoid present in pine wood, is another promising compound that has demonstrated significant anti-inflammatory and antiproliferative properties. These effects are partly attributed to its ability to induce cell cycle arrest and enhance apoptotic pathways in cancer cells (11,12). The wide range of biological activities and pharmacokinetic properties of these compounds make them promising candidates for inhibiting GSTP1 in cancer treatment.

Recent advancements in computational methods, especially molecular docking and ADME (Absorption, distribution, metabolism, and excretion) analysis, have significantly transformed the drug discovery process. Molecular docking allows for the prediction of interactions between small molecules and target proteins, while ADME profiling evaluates the pharmacokinetic properties of a compound, ensuring its effectiveness in biological systems. These techniques are essential for screening extensive natural compound libraries and identifying promising candidates for further experimental testing (13,14).

In this study, ethacrynic acid was used as a reference inhibitor to assess the binding affinities and docking performance of selected natural compounds. Although ethacrynic acid is a recognized GSTP1 inhibitor, its limitations emphasize the need for alternatives that offer enhanced safety and efficacy (15,16). This study compares ethacrynic acid with compounds like bakuchiol, sesamin, hydroxytyrosol, stigmasterol, and pinosylvin to explore their potential as GSTP1 inhibitors. By combining molecular docking and ADME profiling, it aims to identify natural compounds that could address the limitations of current inhibitors. The findings could lead to safer and more effective GSTP1-targeted cancer therapies and highlight the untapped potential of plant-derived compounds in drug discovery.

Material and Method

Preparation of Ligands

In this study, the molecular structures of several natural compounds, along with the GSTP1 inhibitor

ethacrynic acid, were retrieved from the PubChem database for docking analysis (17). The compounds selected for the study included bakuchiol (PubChem ID: 5468522), sesamin (ID: 72307), hydroxytyrosol (ID: 82755), stigmasterol (ID: 5280794), pinosylvin (ID: 5280457), and ethacrynic acid (ID: 3278). Prior to docking simulations, these molecular structures were energy-minimized using Avogadro software to ensure they adopted energetically favorable conformations, thereby improving the accuracy of the docking process (18).

Docking Procedure

The crystal structure of the Glutathione S-transferase P1 (GSTP1) enzyme was obtained from the Protein Data Bank (PDB) with the ID 2GSS. The structure has a resolution of 1.9 Å and *R-factor and R-free values* of *0.209 and 0.229,* respectively (19). Water molecules and other non-protein components were removed to prepare the structure for docking. Hydrogen atoms were then added, and Gasteiger charges were assigned to the protein to ensure precise docking results. The active site of GSTP1 was determined by examining the binding pocket of ethacrynic acid, a known GSTP1 inhibitor. The coordinates for the active site were defined as follows: x = 9.07595, y = 1.00542, and z = 26.9067. A cubic grid of 15 Å × 15 Å × 15 Å was created around this region to guide the docking simulations. The docking was performed using AutoDock Vina (version 1.2.5), utilizing the Lamarckian Genetic Algorithm with its default settings to compute the binding affinities of each ligand (20,21).

Analysis of Molecular Interactions

After the docking simulations were finished, the binding interactions between GSTP1 and the compounds were analyzed to identify the key interaction types. Using Discovery Studio software, hydrogen bonds, hydrophobic interactions, and other relevant binding interactions were visualized and thoroughly examined (22). These analyses offered valuable insights into the binding mechanisms that govern the interaction between GSTP1 and the selected compounds.

ADME analysis

The pharmacokinetic profiles of the selected compounds and ethacrynic acid were assessed using the SwissADME online tool (http://www.swissadme.ch/), which provides insights into essential ADME parameters for drug development. Molecular structures of the compounds were obtained from the PubChem database in SMILES format and entered into the SwissADME platform. The tool generated predictions for key factors such as absorption (e.g., gastrointestinal absorption and skin permeability), distribution (e.g., blood-brain barrier permeability and P-glycoprotein interaction), metabolism (e.g., cytochrome P450 enzyme interactions), and excretion (e.g., water solubility). Additionally, lipophilicity (LogP and LogD), bioavailability, and adherence to drug-likeness criteria like Lipinski's Rule of Five were assessed. The results, including bioavailability radar plots and medicinal chemistry features, were analyzed to identify compounds with favorable pharmacokinetic profiles, focusing on those with optimal absorption, solubility, and metabolic stability for further investigation.

Statistical Analysis

Energy minimization of the compounds was performed using Avogadro, which employs the default MMFF94 force field to optimize molecular geometries, ensuring stable conformations prior to docking. This step includes default statistical methods to assess the stability and energy profiles of the minimized structures. Docking simulations were carried out with AutoDock Vina, utilizing its standard Lamarckian Genetic Algorithm to calculate binding affinities based on both energy and geometric complementarity. The docking process also incorporated default statistical approaches to evaluate the reliability and significance of the calculated binding affinities. The docking results were visualized using Discovery Studio, which provides standard features to assess binding affinities and interaction frequencies. This software enables the identification of critical interactions, such as hydrogen bonds and hydrophobic contacts, while applying default statistical analyses to offer insights into the distribution and significance of these interactions.

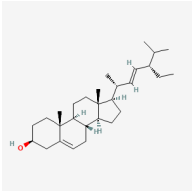
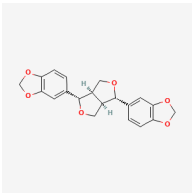
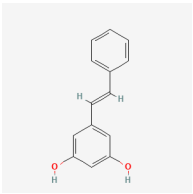
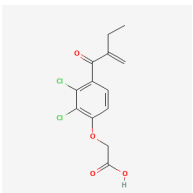
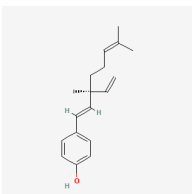
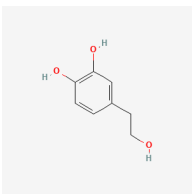
Results

Evaluation of Molecular Docking Analysis

The molecular docking study demonstrated that the natural compounds interact with the GSTP1 protein at varying strengths, as detailed in Table I.

Binding energies, measured in kcal/mol, indicate the interaction strength, with more negative values corresponding to stronger binding affinities. The inhibition constant (Ki) serves as a key parameter to evaluate the compounds' ability to inhibit GSTP1 activity, with lower Ki values indicating higher potency and stronger affinity for the target protein. The Ki values were derived from the binding energy (ΔG) using the formula Ki = eΔG/RT, where R is the universal gas constant (1.985×10^{-3} kcal mol^{-1} K^{-1}), and the temperature (T) was set at 298.15 K.

Table I. Binding Energies of Photosensitizer Compounds and Ethacrynic Acid to GSTP1

Compounds	Molecular structure	Binding energy (kcal/mol)	Calculated *Ki* (μM)
Stigmasterol		-9.2	0.18
Sesamin		-8.2	0.96
Pinosylvin		-7.1	6.16
Ethacrynic acid		-6.7	12.12
Bakuchiol		-6.5	16.99
Hydroxytyrosol		-4.9	253.68

The molecular docking analysis identified stigmasterol and sesamin as the most effective GSTP1 inhibitors among the compounds tested. Stigmasterol exhibited the most potent binding affinity (-9.2 kcal/mol) and the lowest inhibition constant (0.18 μM), followed closely by sesamin (-8.2 kcal/mol, Ki: 0.96 μM). Pinosylvin also displayed moderate inhibitory potential (-7.1 kcal/mol, Ki: 6.16 μM). These results suggest that these natural compounds may offer advantages over ethacrynic acid, a known GSTP1 inhibitor (-6.7 kcal/mol, Ki: 12.12 μM). In comparison, bakuchiol and hydroxytyrosol showed weaker interactions, with hydroxytyrosol being the least effective. These findings highlight stigmasterol and sesamin as promising candidates for further research into GSTP1-targeted therapies.

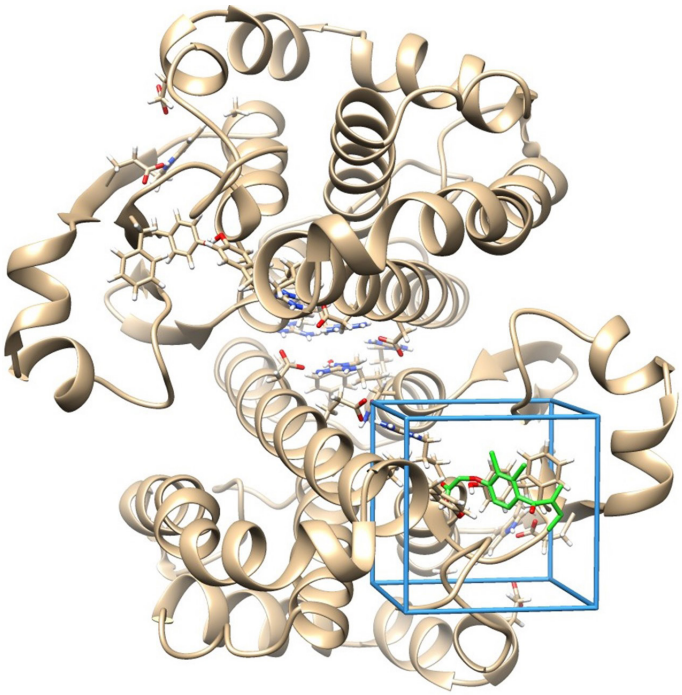


Figure I. 3D Binding pose of ethacrynic acid within the active site of GSTP-1 with a 15 Å cubic grid box. The GSTP1 protein structure is depicted in brown while the structure of ethacrynic acid is shown in green.

Spatial Arrangement of Compounds

The docking process of GSTP-1 with ethacrynic acid was performed using a grid box containing the active site of GSTP1 (H-site), as shown in Fig. I. The grid box was positioned around the site where the crystal structure of ethacrynic acid was previously identified in a complex with GSTP-1. A cubic grid box of 15 Å was created around this site, accommodating

the molecular size of the compounds. Subsequently, all compounds were docked within this grid box.

Furthermore, the spatial arrangement of each compound within the active site of GSTP1 is illustrated in Fig. II. This figure provides a visual overview of the docking results, highlighting how each compound is positioned in relation to the active site. These binding patterns provide insight into how each compound may interact with GSTP1, impacting their potential efficacy in cancer therapy applications targeting this enzyme.

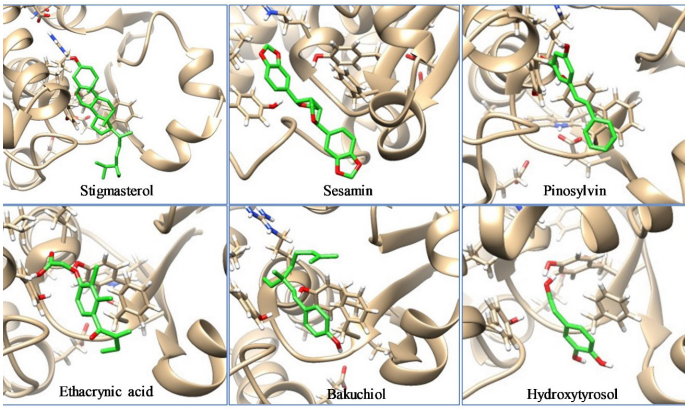


Figure II. The spatial positioning of compounds within the active site of GSTP1. The GSTP1 protein structure is depicted in brown, while the structures of the compounds are shown in green.

Interaction Profiles

The interaction profiles of the compounds with the active site of GSTP1 provide valuable insights into their binding mechanisms and potential as inhibitors (Fig. III). The 2D interaction diagrams illustrate the binding interactions between GSTP1 and four compounds: Stigmasterol (a), Sesamin (b), Pinosylvin (c), and Ethacrynic acid (d). In panel (a), Stigmasterol forms significant van der Waals interactions with residues such as VAL A35 and TYR A7, as well as a pi-alkyl interaction with PHE A3. Additionally, there is an unfavorable donor-donor interaction between ARG A13 and the compound. In panel (b), Sesamin exhibits similar van der Waals interactions with key residues like VAL A10 and TYR A8, and forms a pi-pi stacked interaction with PHE A8. Conventional hydrogen bonds are also observed with residues such as TYR A108. Panel (c) shows Pinosylvin interacting with TYR A7 through a conventional hydrogen bond, while also forming pi-anion interactions with PHE A8 and additional van der Waals contacts. Lastly, in panel (d), Ethacrynic acid demonstrates a range of interactions, including multiple van der Waals contacts with residues such as ARG A19 and ILE A104, as well as pi-anion interactions with the aromatic ring of PHE A8. These interaction profiles highlight the specific binding modes and affinities of each compound with GSTP1, which can contribute to their inhibitory potential.

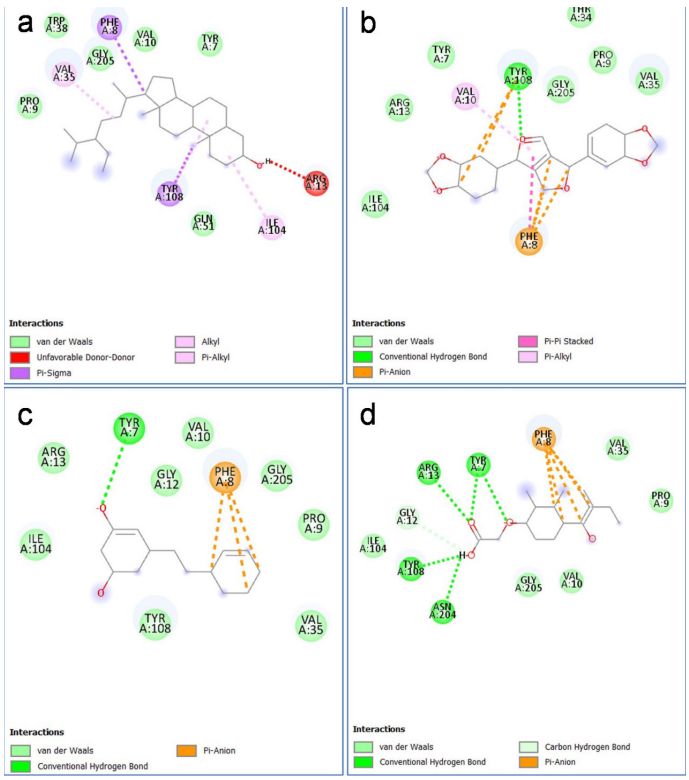


Figure III. 2D Interaction diagrams of top hits and reference compound with GSTP1 protein: (a) Stigmasterol (b) Sesamin (c) Pinosylvin and (d) Ethacrynic acid.

Evaluation of Physicochemical Properties and Pharmacokinetic Profiles

The properties of the compounds with the highest binding energies to GSTP1 were evaluated to gain a better understanding of their potential as inhibitors. This analysis focused on key factors such as molecular size, hydrophobicity, and functional group interactions, which are crucial for determining their binding affinity and overall drug-likeness. By examining these characteristics, we can determine the most promising candidates for further investigation and optimize their potential as therapeutic agents targeting GSTP1

Figure IV presents radar plots and pharmacokinetic data for three compounds: Stigmasterol, Sesamin,

and Pinosylvin. In the radar plots, the red lines represent the compounds' profiles, while the pink shaded areas denote optimal ranges for drug-likeness. Stigmasterol exhibits favorable lipophilicity (LIPO) but shows deficiencies in solubility (INSOLU), suggesting challenges in its bioavailability. Its gastrointestinal (GI) absorption is low, and it does not cross the blood-brain barrier (BBB). Sesamin, on the other hand, demonstrates balanced properties with high GI absorption and BBB permeability, aligning well within the optimal zones for most parameters except solubility. Pinosylvin shows strong flexibility (FLEX) and size (SIZE) attributes but struggles significantly in solubility and saturation (INSATU), despite high GI absorption and BBB permeability. The pharmacokinetics table highlights that none of the compounds act as P-glycoprotein (P-gp) substrates, but Sesamin and Pinosylvin have notable inhibitory effects on CYP450 enzymes (e.g., CYP2C19 and CYP2D6), indicating potential drug-drug interaction risks. Pinosylvin has the highest skin permeability (Log Kp: -5.12 cm/s), followed by Sesamin (-6.56 cm/s) and Stigmasterol (-2.74 cm/s), emphasizing variability in dermal absorption potential. These findings suggest that while each compound has distinct advantages, solubility and enzyme interaction risks remain key considerations in their development as drug candidates.

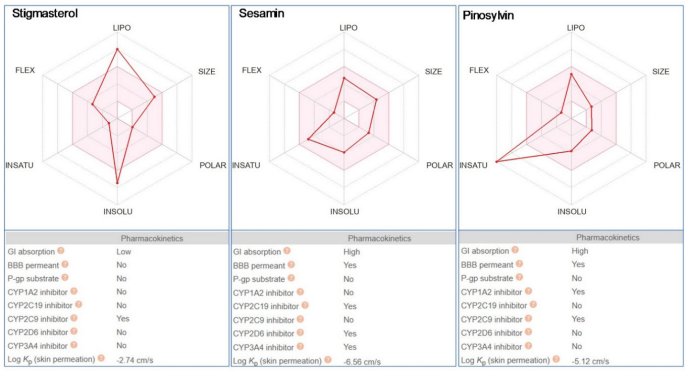


Figure IV. Comparative analysis of physicochemical properties and pharmacokinetics of stigmasterol, sesamin, and pinosylvin

Table II presents the analysis of three natural compounds based on Lipinski's Rule of Five, which evaluates their potential for good oral bioavailability by examining specific molecular properties. Stigmasterol, with a molecular weight of 412.69 g/mol, a LogP of 6.62, one hydrogen bond donor (HBD), and one hydrogen bond acceptor (HBA), has five rotatable bonds and violates one rule due to its high LogP value exceeding the threshold of 5. Sesamin, with a molecular weight of 354.35 g/mol, a LogP of 1.98, no HBDs, six HBAs, and two rotatable bonds, meets all criteria with no violations. Similarly, Pinosilvin complies fully with the rules, featuring a molecular weight of 212.24 g/mol, a LogP of 2.87, two HBDs, two HBAs, and two rotatable bonds. This analysis highlights that while Sesamin and Pinosilvin align with Lipinski's criteria, Stigmasterol may face limitations due to its higher lipophilicity.

Table II. Lipinski's Rule of Five Analysis of Natural Compounds

Compound	MW (g/mol)	LogP	HBD	HBA	Rotatable Bonds	Rule Violations
Stigmasterol	412.69	6.62	1	1	5	1 (LogP>5)
Sesamin	354.35	1.98	0	6	2	0
Pinosilvin	212.24	2.87	2	2	2	0

Abbreviations: MW, Molecular Weight (≤500 g/mol); LogP, Partition coefficient (≤5); HBD, Hydrogen Bond Donors (≤5); HBA, Hydrogen Bond Acceptors (≤10); RB, Rotatable Bonds (≤10).

Discussion

This study has provided valuable insights into the potential of natural compounds as GSTP1 inhibitors, highlighting their role in combating drug resistance, a major challenge in cancer therapy. Through molecular docking and ADME profiling, stigmasterol and sesamin emerged as the most promising candidates, demonstrating superior binding affinities and inhibition constants compared to the reference compound ethacrynic acid. These findings underscore the growing interest in leveraging plant-derived molecules for targeted cancer treatment.

GSTP1, as a key enzyme in detoxification pathways, plays a critical role in neutralizing reactive oxygen species (ROS) and conjugating glutathione to toxic substrates, thereby reducing chemotherapy efficacy in cancer cells (23,24). The docking studies revealed that stigmasterol binds strongly to the active site of GSTP1, with a binding energy of -9.2 kcal/mol and an inhibition constant (Ki) of 0.18 µM. This potency can be attributed to its steroidal structure, which facilitates optimal spatial orientation and robust interactions

with key residues such as VAL A35 and TYR A7. These findings suggest that stigmasterol is not only a potent inhibitor but also an adaptable molecule capable of engaging GSTP1's active site through multiple binding modes. Stigmasterol exhibits a single Lipinski violation due to its high lipophilicity, which may affect its aqueous solubility while enhancing membrane permeability. Despite its relatively low oral bioavailability, stigmasterol's therapeutic potential remains promising, particularly when paired with optimized pharmaceutical formulations. Its flexible molecular structure, characterized by a higher number of rotatable bonds, may enable diverse binding modes with target proteins. Research by Rolta et al. highlights stigmasterol's inhibitory effect on HPV proteins, suggesting its potential as an anticancer agent, especially for localized applications or with enhanced bioavailability (25). Strategies such as nano-carrier systems, liposomes, or solubility-enhancing complexes could further improve its clinical utility (26).

Sesamin demonstrated promising potential with a binding energy of -8.2 kcal/mol and stable hydrogen bond interactions, such as with TYR A108. Its lignan structure supports these interactions, underscoring the role of molecular features in enhancing affinity for target proteins. Sesamin complies with Lipinski's parameters, indicating favorable drug-like properties and a balanced lipophilicity/hydrophilicity profile. However, its poor water solubility and rapid hepatic metabolism significantly limit its oral bioavailability (27). Despite this, studies have shown that sesamin meets key drug-likeness criteria, including favorable ADME properties, making it a promising therapeutic candidate (28). Potential interactions with CYP3A4 and CYP2D6 enzymes highlight the need for careful consideration of drug-drug interactions in clinical use. To address its bioavailability challenges, innovative delivery systems like SNEDDS have proven effective, paving the way for its optimized therapeutic application (27).

Pinosylvin demonstrated moderate inhibition potential based on its binding energy and Ki value, likely influenced by the phenolic groups in its stilbene structure interacting with GSTP1. Its LogP value indicates moderate lipophilicity, is suitable for passive diffusion across cell membranes, and supports oral absorption. The compound's small size and lack of rule violations further enhance its absorption potential. Additionally, its limited number of rotatable bonds suggests a stable metabolic profile, which is beneficial for maintaining bioavailability (29,30). In contrast, bakuchiol and hydroxytyrosol showed lower inhibition potential. The relatively weak affinity of hydroxytyrosol suggests that the presence of phenolic groups alone is insufficient for strong inhibition.

Ethacrynic acid has long served as a benchmark GSTP1 inhibitor. However, its clinical use is limited due to suboptimal pharmacokinetics and significant side effects (31). The comparatively weaker binding affinity (-6.7 kcal/mol) and higher Ki value (12.12 µM) observed in this study emphasize its inferiority to stigmasterol and sesamin. This discrepancy demonstrates that natural compounds not only match but exceed the inhibitory capacity of synthetic inhibitors. Moreover, the reduced toxicity and multifunctional therapeutic effects of natural molecules further elevate their appeal as candidates for clinical application.

Advancing natural compounds as GSTP1 inhibitors necessitates strategic research efforts. Optimizing formulations through innovative delivery systems like SNEDDS or liposomal methods could enhance the bioavailability and stability of stigmasterol and sesamin, addressing solubility challenges. Molecular dynamics simulations may offer deeper insights into binding stability, while structural modifications of lead compounds could improve their potency and pharmacokinetic properties. Preclinical studies are essential to assess anticancer efficacy, toxicity, and biodistribution, with subsequent clinical trials validating therapeutic potential. Additionally, exploring synergistic combinations with standard therapies could enhance effectiveness, reduce drug resistance, and minimize adverse effects, positioning these compounds as promising candidates for resistant cancer treatments.

This study has some limitations, including the fact that the findings are based on in silico analyses, which provide predictive insights but may require further experimental validation. Additionally, factors such as solubility and potential enzyme interactions, especially for compounds like stigmasterol and sesamin, could influence their practical applicability. Future studies

incorporating experimental approaches could help confirm and extend these results.

Conclusion

This study highlights the promising role of natural compounds like stigmasterol and sesamin as GSTP1 inhibitors, offering a novel avenue for tackling drug resistance in cancer treatment. Their unique biochemical properties and the potential for enhancement through modern pharmaceutical strategies position them as valuable candidates in the fight against resistant malignancies. Beyond their direct therapeutic applications, these compounds underscore the broader significance of nature-derived molecules in innovative drug development. With continued focus on refining their efficacy, safety, and delivery, these agents could significantly impact cancer treatment paradigms, bridging the gap between traditional and advanced therapeutic approaches.

References

1. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 2000;61(3):154-166.

2. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 2003;22(47):7369-7375.

3. Oakley AJ, Bello ML, Battistoni A et al. The structures of human glutathione transferase P1-1 in complex with glutathione, and various inhibitors at high resolution. Journal of molecular biology 1997;274(1):84-100.

4. Mehra A, Kaur G, Sethi S et al. Unveiling the potential of Bakuchiol-A comprehensive review on pharmacological activities and therapeutic formulations. Journal of Biologically Active Products from Nature 2024;14(2):134-160.

5. Nizam NN, Mahmud S, Ark SA, Kamruzzaman M, Hasan MK. Bakuchiol, a natural constituent, and its pharmacological benefits. F1000Research 2023;12.

6. Jeng K, Hou R. Sesamin and sesamolin: nature's therapeutic lignans. Current Enzyme Inhibition 2005;1(1):11-20.

7. Majdalawieh AF, Yousef SM, Abu-Yousef IA, Nasrallah GK. Immunomodulatory and anti-inflammatory effects of sesamin: Mechanisms of action and future directions. Critical reviews in food science and nutrition 2022;62(18):5081-5112.

8. Rigacci S, Stefani M. Nutraceutical properties of olive oil polyphenols. An itinerary from cultured cells through animal models to humans. International journal of molecular sciences 2016;17(6):843.

9. Bakrim S, Benkhaira N, Bourais I et al. Health benefits and pharmacological properties of stigmasterol. Antioxidants 2022;11(10):1912.

10. Zhang X, Wang J, Zhu L et al. Advances in Stigmasterol on its anti-tumor effect and mechanism of action. Frontiers in oncology 2022;12:1101289.

11. Bakrim S, Machate H, Benali T et al. Natural sources and pharmacological properties of pinosylvin. Plants 2022;11(12):1541.

12. Goleij P, Sanaye PM, Babamohamadi M et al. Phytostilbenes in Lymphoma: Focuses on the Mechanistic and Clinical Prospects of Resveratrol, Pterostilbene, Piceatannol, and Pinosylvin. Leukemia Research 2024:107464.

13. Adelusi TI, Oyedele A-QK, Boyenle ID et al. Molecular modeling in drug discovery. Informatics in Medicine Unlocked 2022;29:100880.

14. Ferreira LL, Andricopulo AD. ADMET modeling approaches in drug discovery. Drug discovery today 2019;24(5):1157-1165.

15. Ruzza P, Rosato A, Rossi CR, Floreani M, Quintieri L. Glutathione transferases as targets for cancer therapy. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 2009;9(7):763-777.

16. Zhao G, Yu T, Wang R, Wang X, Jing Y. Synthesis and structure-activity relationship of ethacrynic acid analogues on glutathione-s-transferase P1-1 activity inhibition. Bioorganic & medicinal chemistry 2005;13(12):4056-4062.

17. Kim S, Chen J, Cheng T et al. PubChem 2023 update. Nucleic acids research 2023;51(D1):D1373-D1380.

18. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. Journal of cheminformatics 2012;4:1-17.

19. Oakley AJ, Rossjohn J, Lo Bello M, Caccuri AM, Federici G, Parker MW. The three-dimensional structure of the human Pi class glutathione transferase P1-1 in complex with the inhibitor ethacrynic acid and its glutathione conjugate. Biochemistry 1997;36(3):576-585.

20. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2. 0: New docking methods, expanded force field, and Python bindings. Journal of chemical information and modeling 2021;61(8):3891-3898.

21. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry 2010;31(2):455-461.

22. Biovia DS. Discovery studio modeling environment. Release San Diego; 2017.

23. Perperopoulou F, Pouliou F, Labrou NE. Recent advances in protein engineering and biotechnological applications of glutathione transferases. Critical reviews in biotechnology 2018;38(4):511-528.
24. Tew KD. Glutathione-associated enzymes in anticancer drug resistance. Cancer Research 2016;76(1):7-9.
25. Salaria D, Rolta R, Mehta J et al. Phytoconstituents of traditional Himalayan Herbs as potential inhibitors of Human Papillomavirus (HPV-18) for cervical cancer treatment: An In silico Approach. PLoS One 2022;17(3):e0265420.
26. Pireddu R, Sinico C, Ennas G et al. Nanocrystals as Tool to Enhance Stigmasterol Oral Bioavailability. Journal of Nanoscience and Nanotechnology 2021;21(5):2946-2948.
27. Wang C-Y, Yen C-C, Hsu M-C, Wu Y-T. Self-nanoemulsifying drug delivery systems for enhancing solubility, permeability, and bioavailability of sesamin. Molecules 2020;25(14):3119.
28. Kumar A, Mishra DC, Angadi UB, Yadav R, Rai A, Kumar D. Inhibition potencies of phytochemicals derived from sesame against SARS-CoV-2 main protease: A molecular docking and simulation study. Frontiers in chemistry 2021;9:744376.
29. Fedi A, Vitale C, Ponschin G, Ayehunie S, Fato M, Scaglione S. In vitro models replicating the human intestinal epithelium for absorption and metabolism studies: A systematic review. Journal of Controlled Release 2021;335:247-268.
30. Stoner CL, Cleton A, Johnson K et al. Integrated oral bioavailability projection using in vitro screening data as a selection tool in drug discovery. International journal of pharmaceutics 2004;269(1):241-249.
31. Dong S-C, Sha H-H, Xu X-Y et al. Glutathione S-transferase π: A potential role in antitumor therapy. Drug design, development and therapy 2018:3535-3547.