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Original article

Exploring Ginsenosides for Drug Development: Molecular Docking and In Silico Analysis of p53-Mediated Tumor Suppression in Breast Cancer

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Abstract

Breast cancer remains one of the main causes of illness and death linked to cancer worldwide, which highlights the need for better treatment options. Even with the development of targeted therapies, problems like drug resistance, side effects, and high costs show the importance of finding new medicines that are both safe and effective. For many years, natural sources have provided useful compounds for drug discovery, with a wide variety of chemical structures that may help fight cancer. Among these, ginsenosides—special compounds found in the Panax plant genus—have attracted a lot of attention because they have multiple health benefits, including anti-inflammatory, antioxidant, and cancer-fighting properties. Recent studies suggest that certain ginsenosides, such as Rg3, Rh2, and Compound K, have strong anti-cancer effects by affecting key pathways that control how cancer cells grow and survive. One such pathway is the phosphoinositide 3-kinase (PI3K) pathway, which plays a major role in the development of breast cancer by controlling cell growth, metabolism, and resistance. Targeting this pathway is a promising treatment strategy, as shown by FDA-approved drugs like copanlisib. However, there is still a need to find natural inhibitors of the PI3K pathway that are just as effective but have fewer side effects. In this study, we look at how different ginsenosides interact with the PI3K pathway using advanced computer models and tools like CB-Dock2 and BIOVIA. By understanding how well these compounds bind and how they fit together, we aim to find strong ginsenoside candidates that could offer an affordable and natural cancer treatment. We also examine the challenges related to how well these compounds are absorbed and dissolved in the body, so we can improve their properties to make them better medicines.

Keywords: ginsenosides, breast cancer, PI3K signaling pathway, molecular docking, natural inhibitors, drug development.

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Introduction

Breast cancer is one of the most common cancers worldwide, typically originating in either the ducts (ductal carcinoma) or lobules (lobular carcinoma) or breast tissue (Christgen et al. 2018). A major risk factor for hereditary breast cancer is genetic mutation, particularly in the BRCA1 and BRCA2 genes, which play a crucial role in DNA repair. Mutations in these genes significantly increase cancer susceptibility (Mehrgou & Akouchekian, 2016; Qu et al., 2023). Hormonal factors also contribute to breast cancer risk. Prolonged exposure to estrogen and progesterone due to early menstruation, late menopause, or long-term hormone replacement therapy can elevate the risk (Temkin et al., 2019). Additionally, lifestyle factors such as alcohol consumption, obesity, and physical inactivity further increase susceptibility (Feng et al., 2018).

Current breast cancer treatment has advanced significantly, with a multimodal approach including surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy. Surgery (lumpectomy or mastectomy) often serves as the first line of defense, followed by radiation or chemotherapy to reduce recurrence risks (Pestana & Ibrahim, 2020). Targeted therapies like HER2 inhibitors and hormone therapies have improved outcomes, while immunotherapies such as checkpoint inhibitors are emerging as promising treatments (Mercogliano et al., 2023). However, challenges remain, including drug resistance, tumor heterogeneity, and the side effects of treatments, such as cardiotoxicity from some targeted drugs. Additionally, Personalized medicine, based on genetic profiling, offers hope, but access and affordability remain significant barriers, especially in low-resource settings. Nowadays, a natural substitution to minimize these challenges is still under research (Parikesit et al., 2024). One of the natural substances is ginseng, commonly found in several parts of Asia, such as Japan, Korea, and China, with the most well-known being *Panax ginseng Meyer (Fang et al., 2023)*. The natural substances in ginseng called ginsenosides are well-known for varying therapeutic qualities. They are categorized according to their chemical makeup and effects into many sorts. Rg3 is a ginsenoside that has anti-inflammatory and anti-cancer effects. Rh2 is known for its immune-boosting effects and its ability to fight cancer. Digesting ginsenosides forms compound K, which has been associated with anti-inflammatory and anti-cancer properties. F2 is an additional ginsenoside metabolite that contains anti-cancer potential and improves immunological function. These substances add to the many medicinal advantages of ginseng (Valdes et al., 2023).

In this in-silico analysis, the ginsenoside models will target the phosphoinositide 3-kinase (PI3K), which are protein kinases involved in the PI3K/AKT pathway. It is known that the upregulation of PI3K is associated with breast cancer progression & tumorigenesis and resistance

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towards chemotherapy and hormone therapy (Zhang & Richmond, 2021; Scappaticcio et al., 2023). Thus, inhibition of the PI3K/AKT pathway with the use of Ginsenosides is targeted in this study (Luthfiana et al., 2023). Thus, this study aims to contribute to the development of personalized medicine for breast cancer treatment by identifying specific ginsenosides that target p53 and inhibit breast cancer cell growth.

Methods

Data Retrieval

A total of 21 compounds were downloaded from PubChem in SDF format. The ginesonside variant was used as the filter, and the compounds that have potential based on research and studies were selected. The PI3K (PDB ID:1H9O) structure was obtained from the RCSB protein data bank and downloaded in PDB format. The Copanlisib compound, which acts as the control, was obtained from PubChem in SDF format.

Data Preparation

The ligands downloaded in SDF format will first be converted with the BIOVIA Discovery Studio to minimize and create a forcefield of CHARMm and particle charge of MMFF94. The P13K was run through the Pymol program to remove the water molecule.

CB-Dock2, Discovery Studio

The P13K and each ligand are input into CB-Dock2 to assess the pocket binding affinities. CB-Dock2 is a blind docking tool that facilitates predicting protein-ligand interactions without prior knowledge of the binding site location on the protein structure. It employs a protein-surface-curvature-based cavity detection method called CurPocket to facilitate accurate prediction of potential binding sites on the protein structure (Liu et al., 2022).

To begin using CB-Dock2, upload the protein structure in PDB format. The ligand is in SDF format to ease analysis. CB-Dock2 automates the processing of the ligand by adding hydrogen atoms and partial charges, generating an initial 3D conformation using RDKit. The protein verifies and supplements missing side chains and hydrogen atoms, alerts users to any absent residues, and eliminates co-crystallized water molecules and nonstandard groups (Liu et al., 2022). Using a cavity detection algorithm, CB-Dock2 predicts potential binding sites on the protein surface, optimizing ligand placement for accurate docking. Upon completion, detailed results encompassing binding interactions, affinities, and ligand conformations can be visualized for further analysis. Then, docked ligands and proteins from CB-Dock2 were brought to BIOVIA Discovery Studio again for visualization and merging into a PDB file for the type of interactions that occur.

Drug Toxicity Screening

All the ginsenoside compounds and control underwent analysis to evaluate their pharmacokinetic and toxicity profiles. The ADME properties, including absorption, distribution, metabolism, and excretion, were predicted using SwissADME (http://www.swissadme.ch/). The three main parameters to be measured were the molecular weight, water solubility, and Gastrointestinal (GI) absorption. Molecular weight refers to the total mass of a molecule, expressed in Daltons (Da). Water solubility indicates the compound's ability to dissolve in water, which is essential for drug absorption and distribution. Furthermore, GI absorption predicts the compound's ability to be absorbed through the human intestinal tract, which is crucial for oral bioavailability.

All the ginsenoside compounds and control were further tested for their toxicity levels through the ProTox 3.0 software available on the website (https://tox.charite.de/protox3/index.php?site=home).
The two main parameters to be assessed were the predicted LD50 and predicted toxicity class. LD50 refers to the dose of a compound that is lethal to 50% of the experimental animals exposed, usually expressed in mg/kg. LD50 values indicate the toxicity of a substance; a lower LD50 value means higher toxicity (Saganuwan, 2017). Moreover, based on the LD50, a toxicity class was predicted, which was defined according to the globally harmonized system of classification and labeling of chemicals (GHS).

Molecular Dynamics Simulation

The molecular docking result from CB-Dock2 was uploaded into CABS-flex to assess the molecular dynamics. CABS-flex is a molecular dynamics tool used to predict protein backbone flexibility and dynamics (https://biocomp.chem.uw.edu.pl/CABSflex2). It utilizes a Monte Carlo-based simulation to explore the conformational flexibility of proteins while maintaining a simplified representation of the protein structure (Kuriata et al., 2018). The analysis tools were then used to evaluate various parameters, such as root mean square deviation (RMSD) and root mean square fluctuation (RMSF), providing insights into the stability and flexibility of ginsenosides interactions as an inhibitor.

Results and Discussion

Protein preparation

The PI3K molecule was chosen based on the studies of Qu et al., 2023 (PDB ID: 1H9O), which have shown a promising result for analyzing the binding sites that could be exploited for drug development. Figure 1 shows the 1H9O protein that was cleaned in PyMol by removing the water molecule. After that, the cleaned 1H9O was saved and would be used later as a standard for molecular docking. After reviewing the sequence cluster, it is evident that it has a 100% identity, which further confirms that 1H90 was indeed a PI3K molecule. It has also been proven to be derived from Homo sapiens, which requires the criteria to be used for the molecular simulation protocol. The use of a protein sequence cluster with a 100% identity is highly crucial as this would prevent the yield of inaccurate results which were consequent of different protein 3D-morphology, particularly in the active site due to mutations, affecting the integrity of con-

formation, structural stability, and binding interactions of the protein towards the ligands of interest (Pozzati et al., 2022; Song et al., 2024).

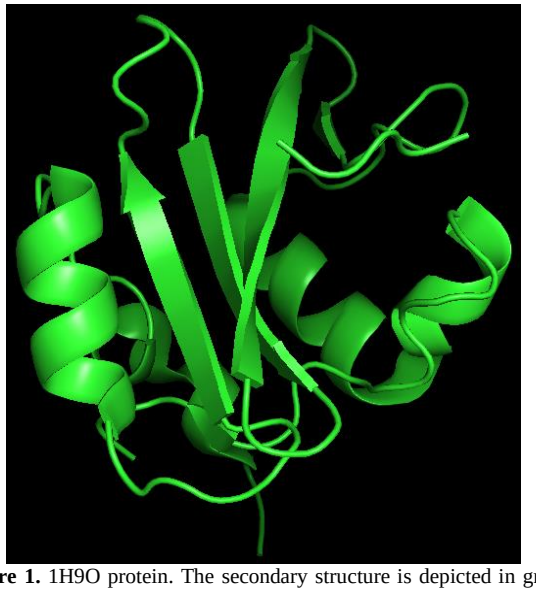

Figure 1. 1H9O protein. The secondary structure is depicted in green color.

Furthermore, 100% sequence identity may allow for reliable molecular dynamics simulations due to the lack of deviation in protein structure that is significant in pose prediction as well as binding affinity, which is highly crucial for drug development (Debnath & Ghosh, 2024). In addition to this percentage identity, the 1H9O protein was also cleaned by the removal of unnecessary water molecules in the protein structure. This step was highly crucial to ensure an enhanced scoring accuracy, as the amount or specific location of water molecules may affect the energy and binding interactions between the protein and ligands, thus affecting the obtained scores of the docking procedure (Kochnev et al., 2024; Xiao et al., 2018). Thus, optimization of the results above can be ensured by selective removal of water molecules in this residue.

Ligand Preparation

Ginsenosides are natural compounds derived from the plant genus Panax (ginseng), which are known for their beneficial effects, including anti-inflammatory and anti-cancer properties (Ratan et al., 2020; Jakhmola et al., 2023). Ginsenosides mainly affect the activity of AMPK/AKT and PI3K/AKT pathways, which are both usually expressed in breast cancer, making them relevant for comparison with copanlisib (Ghafouri-Fard et al., 2022). In this study, A total of 21 ginsenoside variants have been chosen based on a literature review and scientific literature with a promising result in modulating the PI3K/AKT pathway. In all of the variants below namely, Rg1, Rg3, Rg4, Rg5, Rg6, Rf, Rh1, Rh2, Rh3, Rh4, Rh5, Rh7, Rh8, Rk1, Rk2, Rk3, Rs1, Compound K, F1, F2 and F3, the specific origin & references and smiles were annotated with respect to the ginsenoside molecules (Table 1). The variation of ginsenoside variants allowed for the exploration of different conformations and binding interactions with the protein residue, thus allowing selection of compounds with higher efficacy for drug design by the selection with respect to binding affinity (McGinnity et al., 2025; Hu et al., 2024).

Copanlisib, on the other hand, was used as a control as this drug is a PI3K inhibitor, mainly the PI3K-α and PI3K-δ isoforms, therefore, disrupting the cell survival, proliferation, and increasing apoptotic tendencies of B-cell malignancies (Mensah et al., 2018). Unlike some PI3K inhibitors that are taken orally, copanlisib is administered intravenously, and its dosing schedule is less frequent than oral therapies, which have the potential to improve the patient's compliance (Muñoz et al., 2021). The use of copanlisib as a control in this research is supported by accelerated approval provided by the U.S Food and Drug Administration (FDA), as copanlisib was proven to have significant efficacy in the phase 2 clinical trial demonstrated in the CHRONOS-1 study (FDA, 2017). This was evident as copanlisib displayed an objective

Table 1. The compounds of Ginsenosides from scientific literature

Ginsenosides Variants	Origin	Reference	Pubchem ID
Copanlisib (control)	Developed by Bayer HealthCare Pharmaceuticals and marketed under the brand name Aliqopa	(Markham, 2017)	135565596
Rg1	Founded in Panax ginseng and Panax notoginseng	(Zhao et al., 2014)	441923
Rg3	Generated through steaming or heat processing of ginseng specifically red ginseng	(Jo et al., 2014)	9918693
Rg4	Minor ginsenoside product from steaming or fermentation of ginseng	(Huang et al., 2022)	102004835
Rg5	Found in heat-processed or fermented ginseng	(Jo et al., 2014)	11550001
Rg6	Rare ginsenoside formed via steaming of ginseng	(Piao et al., 2020)	91895489
Rf	Extracted from Panax ginseng roots	(Yang et al., 2014)	441922
Rh1	Found in Panax ginseng and forms during heat treatment	(Kim et al., 2020)	12855920
Rh2	Found in red ginseng or processed through fermentation	(Piao et al., 2020)	119307
Rh3	Minor ginsenoside from heat-treated ginseng	(Ji et al., 2023)	20839223
Rh4	Found in steamed ginseng products	(Wang et al., 2024)	21599928
Rh5	Minor ginsenoside produced during steaming	(Zhang et al., 2020)	10699455
Rh7	Found in heat-processed ginseng	(Chen et al., 2021)	101096472
Rh8	Rare ginsenoside in heat-treated or fermented ginseng	(Tang et al., 2024)	85245726
Rk1	Formed by steaming or high temperature processing of ginseng	(Jo et al., 2014)	11499198
Rk2	Found in steamed or fermented ginseng products	(Piao et al., 2020)	90472238
Rk3	Generated through heat-processing of ginseng	(Huang et al., 2022)	75412555
Rs1	Minor ginsenoside identified in Panax ginseng root	(Piao et al., 2020)	85044013
Compound K	The primary metabolite of ginsenoside Rb1, Rb2, and Rc	(Zhou et al., 2022)	9852086
F1	Derived from Rg1 or Re by enzymatic or acidic hydrolysis	(Fan et al., 2021)	9809542
F2	Formed by enzymatic hydrolysis of Rb1 or Rc	(Song et al., 2017)	9918692
F3	Rare derivative found through fermentation of Panax ginseng	(Piao et al., 2020)	46887678

response rate (ORR) of 60.6%, a partial response (PR) rate of 43.7% and also a complete response (CR) rate of 16.9%. Thus, signifying a great anti-tumor activity as these variables showed a significant number of patients whose tumor shrank with copanlisib treatment (Dreyling et al., 2020).

CB-Dock2

The molecular docking results were assessed using Vina scores, which indicate the binding quality between proteins and small molecules. CB-Dock2 employs a cavity detection algorithm to identify potential binding sites on the protein surface, ensuring that the ligand is directed toward the most likely active sites. The Vina scoring function combines multiple parameters, including steric clashes, hydrophobic contacts, and hydrogen bonds, to provide a comprehensive assessment of binding affinity (Koebel et al., 2016). These interactions are computed and modified according to the number of rotatable bonds, considering the entropic penalties linked to molecular flexibility. In this scoring system, a lower (more negative) value signifies stronger binding (Quiroga & Villarreal, 2016). Thus, a more negative value is preferred. The result is in Table 2 shows a result for the binding affinity with ranges of -5.2 to -7.0, whereas the ginsenoside Rs1 acquired the greatest binding affinity compared to the control, which scored -6.9.

Table 2. Binding affinity and type of ligand-protein interactions of ligands through CB-Dock2 & Discovery studio analysis

Ligand	Binding Affinity (kcal/mol)
Copanlisib (Control)	-6.9
Rg1	-6.0
Rg3	-6.6
Rg4	-6.2
Rg5	-5.9
Rg6	-5.5
Rf	-5.5
Rh1	-5.2
Rh2	-6.2
Rh3	-6.8
Rh4	-5.6
Rh5	-5.7
Rh7	-6.4
Rh8	-5.7
Rk1	-6.3
Rk2	-6.3
Rk3	-5.7
Rs1	-7.0
Compound K	-6.4
F1	-6.2
F2	-6.4
F3	-6.5

As control that is clinically approved as PI3K inhibitor (Dreyling et al., 2020), copanlisib has strong interaction that can be attributed to the formation of multiple stabilising interactions, including hydrogen bonds, alkyl interactions, and van der Waals forces, with residues such as HIS57, PHE69, and LEU98 (Figure 2A). Compound K, which is a biotransformed compound of ginsenoside, elicited weaker interaction and binding due to the diference in the binding sites (Figure 2C). Rh2 and F2 elicit the relatively weak binding because the cavity of the target is buried inside the structure (Figure 2B and D). These residues are important as the key components of the ATP-binding site, emphasizing the compound's efficacy in competitively inhibiting PI3K activity (Zhang et al., 2020). Among the ginsenosides as it can be seen from the Table 2, Rs1 displayed the highest binding affinity with -7.0 kcal/mol, followed by Rh3 with -6.8 kcal/mol, and lastly F3 with -6.5 kcal/mol, suggesting that these compounds may serve as potential competitive inhibitor of PI3K beside copanlisib. The strong docking score of these ginsenosides may have a connection with their molecular size and conformational flexibility (McNutt et al., 2021). Larger ginsenosides may experience steric hindrance, reducing their ability to fit optimally into the binding site (Zhong et al., 2021). On the other hand, smaller or more flexible molecules can adapt their conformation better to the binding side, enabling tighter binding and a more favorable affinity score (Tripathi & Bankaitis, 2017). For instance, smaller ginsenosides, such as Rh3 and F3, have better structural adaptability, allowing them to form stable interactions with key residues. However, despite these advantages, their lower molecular weight may result in weaker hydrophobic interactions compared to larger compounds such as Rs1 (Pan et al., 2018).

Little was known about the ginsenoside ligand Rs1, however Rs1 or otherwise known as β-D-glucopyranoside is a class of naturally phenolic glycoside compounds found in ginseng and this compound has a relative similarity towards another compound found in ginseng, Oleanolic acid 28-O-β-D-glucopyranoside (OAG) which is a pentacyclic triterpenoid class. A recent study conducted by Wang et al., 2024 showed that OAG has a potential to modulate the PI3K/MAPK pathways. In addition to that, OAG was also known to have an anti-inflammatory effect, promote gut microbiota as well as enhancing and maintaining epithelial cell and barrier function in Ulcerative colitis (UC) (Pubchem, n/d; Wang et al., 2024). Furthermore, another study showed that a similar glucopyranoside variant, (Z)-3-hexenyl-β-D-glucopyranoside was known to induce apoptosis and cell growth arrest at the S phase of pancreatic cancer cell line, Panc1, and further analysis have shown an enhanced expression of apoptotic genes such as Casp2 and Bax as well as a decrease expression of antiapoptotic gene, Bcl-2 (Zaher et al., 2023; Antonius et al., 2022). Thus, with this supporting literature, further research about Rs1 must be done in order to understand its efficacy as a potential candidate for cancer treatment.

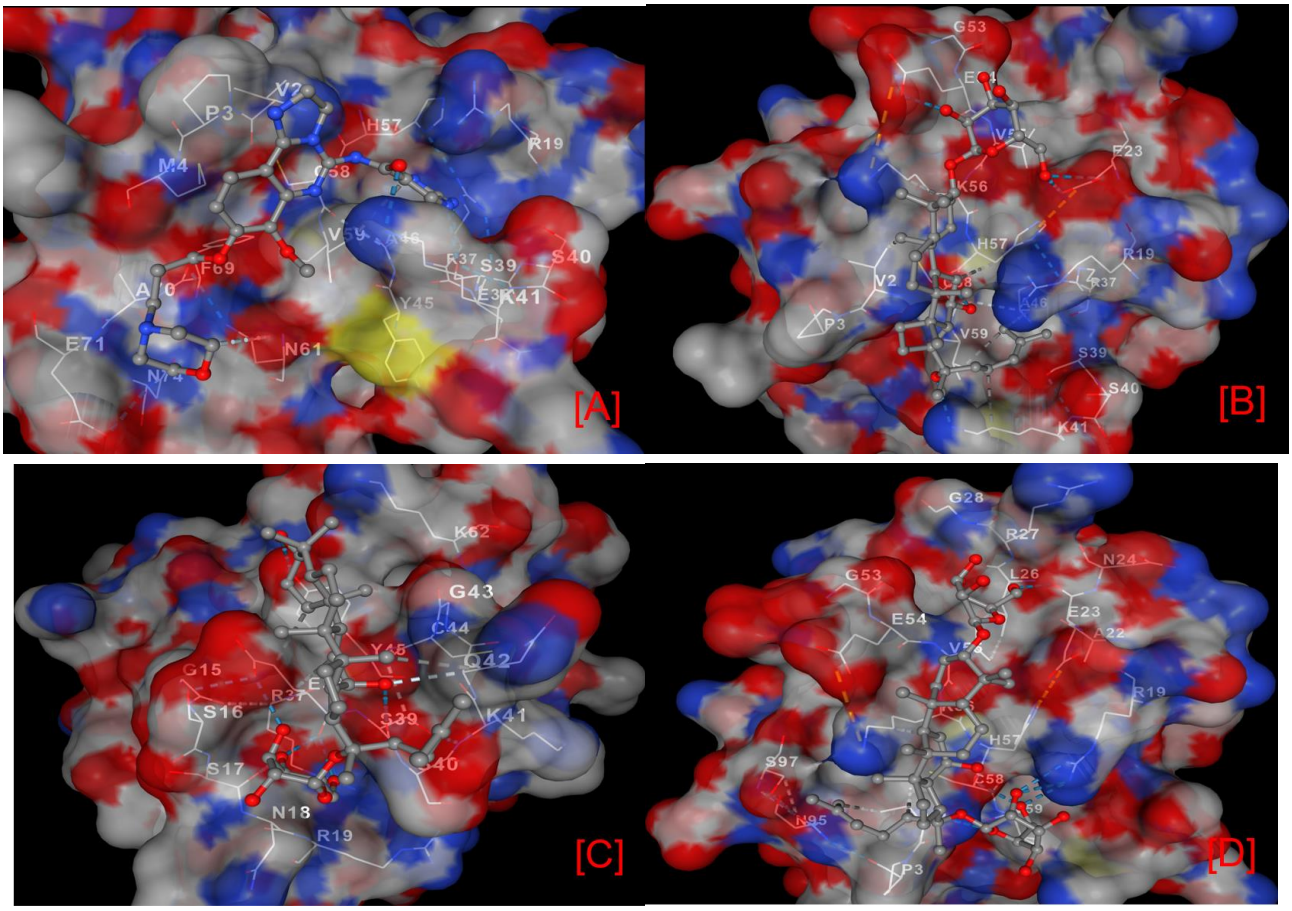

Figure 2. (A) Molecular docking of Copanlisib ligand, (B) Rh2 , (C) Compound K,(D) F2

Aside from Rs1, Rh3 and F3 was also known to have significant potential as a cancer treatment. Rh3 was known to have a great antitumor effect towards human tumors whereas this ligand was known to lead to ferroptotic cell apoptosis through of Stat3/p53/NRF2 mechanisms with minimal adverse effect towards normal cells, whereas this was achieved by several mechanisms such as, GSDMD-dependent pyroptosis activation, NLRP2 upregulation through H0-1 downregulation as well as, suppression of SLC7A11 which depletes glutathione thus causing an upregulation in reactive oxygen species, malondialdehyde and iron (Wu et al, 2023). In addition to Rh3, F3 was also known to exert anticancer effects towards immortalized breast cancer cell lines, MDA-MB231 and murine 4T1, by disruption of the metastatic mechanisms (Baraya et al., 2022). Furthermore, F3 was said to induce DNA damage by stimulating DNA strand breaks and γ-H2AX activation that may lead to MAPK pathway activation, which leads to MCF-10A breast cancer cell line apoptosis, as well as induce autophagy by initiating autophagosome formations and upregulation of LC3-II concentrations (Zhu et al., 2019).

SWISS ADME Results

Screening performed in SWISS-ADME analyzed the selected ginsenosides variants for key pharmacokinetic (absorption, distribution, metabolism, excretion) and physicochemical (toxicities) properties of drugs/ligands. In Table 3. The molecular weight of all ginsenoside variants ranges from 604.86 g/mol to 1121.31 g/mol, exceeding the normal size of PI3K inhibitors and the optimal size for drug-like properties. This was supported by the "Lipinski's rule of five" which states that drugs with a partition coefficient (logP) of higher than 5, hydrogen donor groups of more than 5, hydrogen acceptor groups of higher than 10 and most importantly, the molecular weight of more than 500 g/mol are said to have impaired permeation as this implored that the drug did not have a balance hydrophilic-lipophilic characteristics (Roskoski et al., 2019).

Table 3. The compounds of Ginsenosides result from SWISS-ADME

Ligand	Molecular Weight (g/mol)	Water Solubility	GI Absorption
Copanlisib (Control)	480.52	Soluble	Low
Rg1	801.01	Moderately Soluble	Low
Rg3	785.01	Poorly Soluble	Low
Rg4	767.00	Poorly Soluble	Low
Rg5	767.00	Poorly Soluble	Low
Rg6	767.00	Poorly Soluble	Low
Rf	801.01	Moderately Soluble	Low
Rh1	638.87	Poorly Soluble	Low
Rh2	622.87	Poorly Soluble	Low
Rh3	604.86	Poorly Soluble	Low
Rh4	620.86	Poorly Soluble	Low
Rh5	652.90	Poorly Soluble	Low
Rh7	636.86	Moderately Soluble	Low

Rh8	636.86	Moderately Soluble	Low
Rk1	767.00	Poorly Soluble	Low
Rk2	604.86	Poorly Soluble	Low
Rk3	620.86	Poorly Soluble	Low
Rs1	1121.31	Moderately Soluble	Low
Compound K	622.87	Poorly Soluble	Low
F1	638.87	Poorly Soluble	Low
F2	770.99	Poorly Soluble	Low
F3	770.99	Moderately Soluble	Low

Additionally, Egan's rules stated that for oral drugs to be absorbed optimally, the general range of molecular weight would be 200 to 600 Daltons (equivalent to g/mol) (Rai et al., 2023). It was also crucial to note that most PI3K inhibitors attain a molecular weight of below 500 g/mol, whereas Idelalisib, ZSTK474, NVP-BEX235, and Wortamannin have a molecular weight of 415.42 g/mol, 417.41 g/mol, 469 g/mol, and 346.4 g/mol, respectively (Graf & Gopal., 2016; NCBI., 2025). Concerning the control, the ligands Rg1, Rf, Rh7, and Rh8 were indicated with favorable solubility as seen in the water solubility profiles; this is highly critical for bioavailability as higher solubility favors greater drug diffusion rate, which allows sufficient drug concentration and impact at the site of action (Abuzar et al., 2018).

However, as seen in Table 3, the gastrointestinal (GI) absorption analysis revealed low potential for effective absorption, thus decreasing the ligands and control suitability for oral administration. The low GI absorption results of these ligands were possibly due to the high molecular weight of these ligands, whereas certain physicochemical properties such as solubility, particle size, chemical form, and dosage formulation may affect the absorption rate of certain drugs (Luo et al., 2021). Thus, in order to ensure adequate GI absorption, several mechanisms can be applied towards the drug design of these organic ligands, such as site-specific drug administration, optimization of drug release rate, usage of prodrugs, drug vehicle optimization, and possible modification of GI drug metabolism (Alqahtani et al., 2021).

With several factors to consider, such as molecular weight, water solubility, and GI absorption, it can be observed that Rh7 and Rh8 ligands are the best ginsenosides to be considered for breast cancer treatment. In addition, this observation was supported by the fact that Rh7 has a regulatory property in a long non-coding RNA (lncRNA) responsible for various cancers called ILF3-AS1; thus, the downregulation of ILF3-AS1 decreases cancerous cell viability and apoptosis through regulation of miR-212 expression, which cause a downstream effect towards SMAD1 expression (Chen et al., 2021). Furthermore, an in vivo study has shown that ILF3-AS1 is linked to metastasis, vascular invasion, and lymph node metastasis, and thus, the downregulation of ILF3-AS1 would suppress migration, colony-formation, and metastasis mechanisms in malignant cancer cells (Hong et al., 2021; Widyananda et al., 2021). Little was known about the efficacy of Rh8; however, further studies would need to be done regarding the role in cancer mechanisms.

Toxicity Analysis

The toxicity analysis of ginsenoside compound variants is conducted using ProTox3. These tools rely on extensive databases of chemical structures and known toxicities to make predictions (Banerjee et al., 2024). The analysis primarily focused on LD50 values and toxicity classifications. Higher LD50 values indicate lower toxicity; on the contrary, lower LD50 values indicate that the compounds are (Morris-Schaffer & McCoy, 2020). As can be seen from Figure 3, picture A, Copanlisib, as the control compound, has the lowest LD50 value with 2935 mg/kg, which indicates it is more toxic than the majority of ginsenoside compounds that were tested. This is aligned with a journal that was written by Mishra et al. (2021, the known adverse effects of synthetic PI3K inhibitors have severe side effects such as hepatotoxicity, gastrointestinal toxicity, and immunosuppression.

Ginsenosides' high molecular weights and low GI absorption profile might reduce their toxicity since they are less likely to accumulate in sensitive tissues (Zhou et al., 2016). Among the ginsenosides, Rh7 and F3 have higher LD50 values with 6000 mg/kg and 8000 mg/kg, respectively. With these high LD50 values, which indicate very low toxicity, these compounds have favorable toxicity profiles. This characteristic enhances their potential as a safer alternative for long-term therapeutic use. The higher LD50 values for Rh7 and F3 compared to the other ginsenosides could be attributed to several structural and biochemical factors (Xue et al., 2020). For instance, the presence of multiple hydroxyl groups in certain ginsenosides may affect their interaction with other proteins or enzymes in the body, resulting in unintended pharmacological effects (Sarhene et al., 2021).

Based on Table 4. which shows the elaboration of toxicity in terms of its toxicity levels, all the variants including the control exhibit relatively safe toxicity with class 5 predicted toxicity. This is regarded as safe for consumption within appropriate dosages. Furthermore, Rh8 exhibits the highest average similarity score with 83.39% and there's equal in prediction accuracy for Rg1, Rg3, Rg4, Rg5, Rf, Rh1, Rh2, Rh3, Rh4, Rh5, Rh8, Rs1, Compound K, F1, F2, and F3 with 70.97% prediction accuracy. This means that the likelihood of the analyses being true is higher than the others, which scored lower.

Table 4. The Toxicity Analysis of Ginsenosides result from ProTox 3.0 software

Ligand	Predicted Toxicity Class	Average Similarity (%)	Prediction Accuracy (%)
Copanlisib (Control)	5	44.17	54.26
Rg1	5	82.84	70.97
Rg3	5	81.96	70.97
Rg4	5	80.26	70.97
Rg5	5	80.26	70.97
Rg6	5	79.01	69.26
Rf	5	81.96	70.97
Rh1	5	83.04	70.97
Rh2	5	83.04	70.97
Rh3	5	81.23	70.97
Rh4	5	81.23	70.97
Rh5	5	82.84	70.97
Rh7	6	79.63	69.26
Rh8	5	83.39	70.97
Rk1	5	79.01	69.26
Rk2	5	79.31	69.26
Rk3	5	79.31	69.26
Rs1	5	86.76	70.97
Compound K	5	82.84	70.97

F1	5	82.84	70.97
F2	5	82.84	70.97
F3	6	82.04	70.97

Considering all factors in results and discussion, F3 and Rh7 appear to be the most balanced option for further development as cancer treatments due to their low toxicity, moderate solubility, and reasonable binding affinities. Rs1, on the other hand, is a promising candidate as it has strong inhibitory potential. However, it requires structural modification to be able to be consumed as a drug by humans. Further studies need to be conducted to ensure its efficacy in inhibiting P13K, such as downstream analysis, PD/PK assessment, and animal/in vitro studies.

Molecular Dynamics

The molecular dynamics of each ginsenoside are computed with the use of root mean square fluctuation (RMSF) value, which measures the overall change of displacement in each atom with respect to its respective mean position (Li et al., 2024). Generally, lower RMSF values are favorable, as this indicates greater stability with lesser fluctuations in conformations of the atoms/molecules, and is more preferred in drug design as increased stability is critical to maintain the overall function and morphological integrity of the macromolecules. Overall, most of the ginsenosides achieved an RMSF score of below 2.0Å, with Compound K achieving the lowest score of 0.857Å. This indicated that most of the ginsenosides, especially compound K, possess greater stability than the control, copanlisib. As seen in the Table.4, Rg1, Rg3, Rg4, Rg5, Rg6, Rf, Rh1,Rh2, Rh3, Rh4, Rh5, Rh8, Rk1, Rk2, Rk3,Rs1, Compound K, F1, F2 and F3 altogether has RMSF value of lesser than 1.260. Thus, with the copansilib's RMSF value acting as a control, most ginsenosides can be seen as safe due to their atomic stability.

With reference to Table 5, which shows the average root mean square fluctuation value, which quantifies the relative fluctuation/stability of each molecule in the compound (Song et al., 2024). It was crucial to point out that Compound K has the lowest RMSF value and is regarded as the most stable out of all the ginsenosides. These findings were further supported by the results of powder X-ray diffraction (PXRD), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of crystallized Compound K, whereas monohydrate, a component of Compound K, possesses a strong physical attribute; thus, contributing to the overall architecture of the compound (Chen et al., 2019). In addition, numerous animal and clinical studies have reported that Compound K is well tolerated and has little to no malevolent effects on the animal models subjected to these studies (Sharma & Lee, 2020). Recent studies have also shown that Compound K has the strongest anticancer activity when compared to other ginsenosides via the induction of the G1 phase cell cycle arrest as well as suppression of the growth of hormone-independent human breast cancer cells, thus disrupting tumorigenesis and tumor growth (Achmad et al., 2024). Thus, Compound K can be considered to be one of the most promising ginsenosides to be considered as a potential therapeutic agent towards cancer.

Table 5. The average root mean square fluctuation (RMSF) of each ligand

Protein-Ligand structure	RMSF (Å)
Copanlisib (control)	1.260
Rg1	1.184
Rg3	1.170
Rg4	1.044
Rg5	1.132
Rg6	1.103

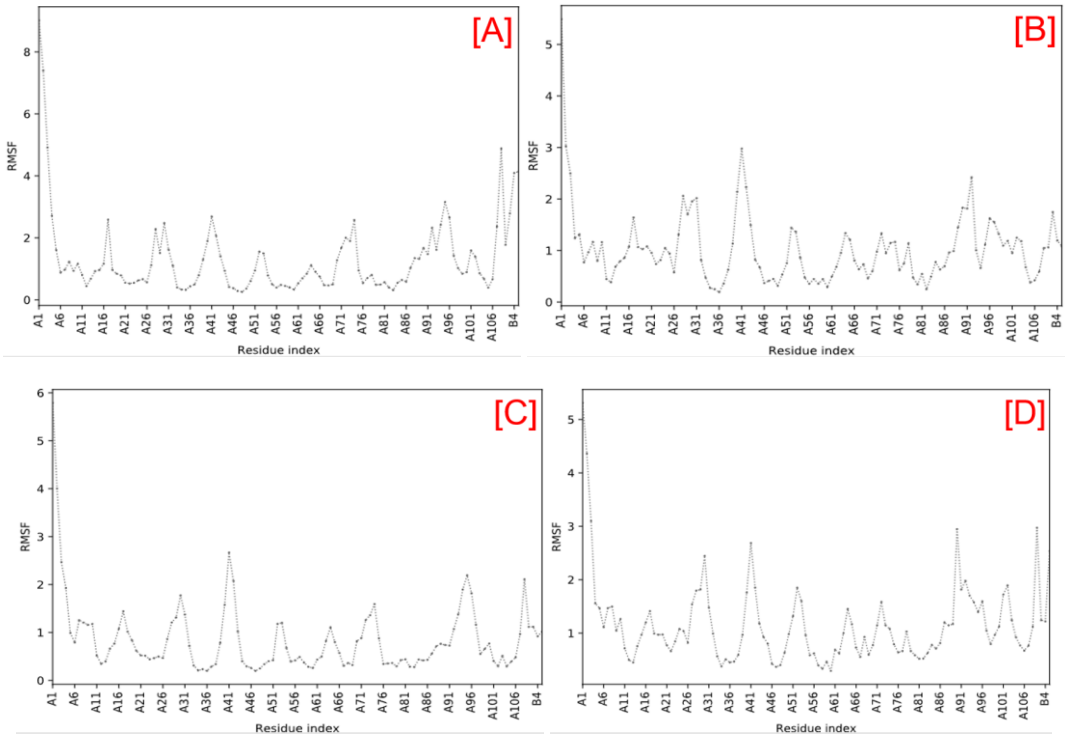

Figure 4. Molecular Dynamics of Copanlisib (Control) (A), Rh2 (B), Compound K (C), F2 (D).

Rf	1.248
Rh1	1.075
Rh2	1.024
Rh3	1.000
Rh4	1.135
Rh5	1.288
Rh7	1.267
Rh8	1.254
Rk1	1.166
Rk2	1.125
Rk3	1.199
Rs1	0.995
Compound K	0.857
F1	1.104
F2	1.131
F3	1.188

Meanwhile, the graphs in Figure 4 displayed slight fluctuations at each amino acid within the structure, but the core residues fluctuate between 0.5 and 2.5 nm, suggesting a relatively stable protein backbone with flexible loop regions when bound to the ligand (Linse et al., 2020). Some flexible loop regions showed higher fluctuations, 2.0-2.5 nm, which is expected. These regions are naturally more dynamic, often playing a role in ligand adaptability (William et al., 2021). This is more likely to correspond to loop regions or solvent-exposed residues that are more flexible than structured α-helices or β-sheets. Overall, the observations indicate that each.

Comparison with Control (Copanlisib)

Copanlisib is a PI3K inhibitor drug that is mainly used for the treatment of certain types of cancer, such as melanoma, lymphoma, and many more. Copanlisib works by inhibiting the PI3K-α and PI3K-δ isoforms, which are expressed in malignant B cells. This can result in inducing tumor cell death by apoptosis and inhibition of malignant B cell proliferation (Huang et al., 2020). Unlike some PI3K inhibitors that are taken orally, copanlisib is administered intravenously, and its dosing schedule is less frequent than oral therapies, which have the potential to improve the patient's compliance (Muñoz et al., 2021). Ginsenosides, on the other hand, are natural compounds derived from the plant genus Panax (ginseng), which are known for their beneficial effects, including anti-inflammatory and anticancer properties (Ratan et al., 2020). Ginsenosides mainly affect the activity of AMPK/AKT and PI3K/AKT pathways, which are both usually expressed in breast cancer, making them relevant for comparison with copanlisib (Ghafouri-Fard et al., 2022).

Mainly, there are factors that can be compared to determine the efficacy of ginsenoside against copanlisib as a treatment, which include the solubility, toxicity, and binding affinity. For solubility, copanlisib is designed to be soluble, which allows for effective IV administration. This ensures that it reaches the bloodstream quickly and has therapeutic effects more quickly (Fergusson et al., 2023). Ginsenosides usually have lower solubility due to the fact that they are less polar than copanlisib. However, some ginsenosides like Rg1 are more soluble due to their increased hydroxyl groups. The solubility of ginsenosides can be increased by fermentation or chemical modifications, which can improve their therapeutic effects (Shen et al., 2023). On the other hand, the toxicity of copanlisib is well-known and studied, as it includes side effects like hypertension and hyperglycemia. The effects are caused by the inhibition of the PI3K pathway, which helps with insulin signaling and metabolic regulation (Cheson et al., 2019). Ginsenosides, however, are considered safer than copanlisib because it has a low toxicity rate. They are generally safe, but high doses can lead to side effects such as GI problems or allergic reactions. Ginsenosides have placed themselves as a potential therapeutic strategy because of their overall safety in both the short and long term (Wang et al., 2021). Lastly, copanlisib shows a high binding affinity score against PI3Kα and PI3Kδ. This interaction leads to an effective inhibition of the pathway that is involved in tumor growth and survival (Vanhaesebroeck et al., 2021). This makes copanlisib one of the best choices for cancer therapy. Ginsenosides also show high binding affinity towards PI3K, where certain ginsenosides, such as Rg3 and Rh2, have the ability to inhibit PI3K activity by competing with ATP or interacting with the subunits. The effectiveness is affected by their structural characteristics, where some ginsenosides could show synergistic effects when combined with other treatments in the same pathway (Yang et al., 2024).

Implications of Findings

This study aimed to assess the potential of ginsenosides Rh2, F2, and Compound K as inhibitors of the PI3K signaling pathway in breast cancer. The molecular docking simulations showed that all three ginsenosides demonstrated strong binding affinities to the ATP-binding site of PI3K, with binding energies comparable to the known inhibitor Copanlisib. Specifically, Rh2, F2, and Compound K formed stable complexes with the enzyme, establishing hydrogen bonds and hydrophobic interactions with key residues in the ATP-binding pocket. The results suggest that these ginsenosides effectively block PI3K activation, a critical step in the PI3K/AKT/mTOR pathway, which is frequently dysregulated in breast cancer. The interactions observed in the docking studies indicate that these compounds could potentially serve as competitive inhibitors of PI3K, thereby reducing cancer cell proliferation and survival. Furthermore, the binding analysis revealed that the ginsenosides interacted with residues that are integral to the ATP-binding site, supporting their role in inhibiting PI3K's enzymatic activity. These findings provide promising in silico evidence for the therapeutic potential of Rh2, F2, and Compound K as PI3K inhibitors for breast cancer treatment.

To further confirm that ginsenosides Rh2, F2, and Compound K could work as PI3K inhibitors in breast cancer, experiments should be done using human breast cancer cell lines like MCF-7 or MDA-MB-231. These tests should focus on how these compounds affect the PI3K/AKT/mTOR pathway by measuring changes in protein levels using methods like Western blotting or quantitative PCR. In addition, cell growth can be tested with assays such as MTT or WST-1, and apoptosis can be measured by flow cytometry to check for changes in caspase activity or Annexin V staining. If the in vitro results are successful, studies in mouse models with

breast cancer are needed to test how well the compounds are absorbed, how they move through the body, and if they cause any harm. This could involve measuring ginsenosides in the blood and checking tumor size reduction, using imaging techniques like bioluminescence imaging. At the same time, the effect of ginsenosides on genes in the PI3K/AKT/mTOR pathway, such as PTEN, pAKT, or mTOR, should be examined to understand how they influence cells. Additionally, testing whether ginsenosides can work together with other PI3K inhibitors, such as BKM120 or PI-103, could help find more effective treatments for breast cancer.

Limitation

The limitation of this research includes its reliance solely on computational predictions without experimental validation. Molecular docking and dynamics simulations cannot fully replicate the complexity of biological systems. Factors such as metabolism, systemic bioavailability, off-target effects, and tumor microenvironment interactions remain unaddressed. Additionally, the poor water solubility, high molecular weight, and low gastrointestinal absorption of most ginsenosides may significantly hinder their clinical applicability without further modification. Therefore, in vitro and in vivo studies, along with structure-activity relationship (SAR) analyses and formulation development, are needed to translate these findings into viable therapeutic candidates.

Conclusion

This study explored the potential of ginsenosides, including Rh2, F3, Compound K, and many others, as natural alternatives to synthetic drugs for breast cancer treatment. The results demonstrated that these compounds possess affinities to the PI3K enzyme, comparable to copanlisib, which is the PI3K inhibitor with lower toxicity, suggesting their capability to inhibit the PI3K signaling pathway effectively. Among the ginsenosides, Rs1 showed the biggest binding affinity, followed by Rh3 and F3, highlighting their potential as inhibitors of PI3K. However, several challenges, such as high molecular weight, low gastrointestinal absorption, and poor water solubility, were identified, which could hinder their clinical application. The findings underscore the promise of ginsenosides as a cost-effective, natural therapeutic option, while also emphasizing the need for molecular modifications to enhance their drug-like properties. To address the challenges highlighted in this study, Future research should also investigate how these compounds influence processes like cell death, cell cycle regulation, and related signaling pathways with the use of in vivo and in vitro analysis in order to understand the impact of these compounds on breast cancer.

This study investigated ginsenosides (e.g., Rh2, F3, Compound K) as natural alternatives for breast cancer treatment. Among them, Rs1, Rh3, and F3 showed the highest binding affinities for the PI3K enzyme, similar to copanlisib, suggesting effective PI3K pathway inhibition. However, high molecular weight, low gastrointestinal absorption, and poor water solubility challenge their clinical use. Promising as cost-effective therapeutics, ginsenosides need molecular modifications and further in vivo and in vitro studies on their effects on cell death, cell cycle, and signaling pathways.

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