Screening of Halogenated Flavonoids from Syzygium Species against PI3Kα for Breast Cancer

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Abstract:

Breast cancer is one of the leading causes of oncological mortality among women worldwide. Hyperactivation of the Phosphoinositide 3-kinase alpha (PI3Kα) signaling pathway plays a fundamental role in tumor cell proliferation, survival, metabolic reprogramming, and development of endocrine drug resistance, rendering it a highly sought-after therapeutic target. Natural flavonoids extracted from *Syzygium* species possess well-documented antioxidant and anti-proliferative capabilities; however, their synthetic modification via strategic halogenation offers a novel chemical space to potentially optimize binding affinities and pharmacokinetic behaviors.

In the present work, a structure-based *in silico* molecular docking screening of several natural *Syzygium* flavonoids—including Luteolin derivatives (*Luteolin 3-methyl ether, Luteolin 4-methyl ether, Luteolin 5-methyl ether, Luteolin 7-methyl ether, and 6-Methoxyluteolin*) and Chrysin derivatives (*Chrysin, Tectochrysin, 6,8-Dimethylchrysin, Chrysin 5,7-dimethyl ether, and Chrysin dimethyl ether*)—alongside their prioritized halogenated analogues (6-bromo-luteolin and 3-iodochrysin) was carried out to evaluate their inhibitory potential against the ATP-binding pocket of the PI3Kα enzyme. Computational virtual screening was performed using AutoDock Vina, and the visualization of the precise macromolecular interaction pathways was evaluated with a visualizer tool.

The binding affinity results were compared directly against Alpelisib, a standard synthetic clinically approved PI3Kα inhibitor. The halogenated derivatives (6-bromo-luteolin and 3-iodochrysin) exhibited exceptional binding energies that surpassed both their non-halogenated parent natural compounds and the standard drug, primarily due to the creation of stable, directional halogen bonds alongside conventional hydrogen-bonding cascades and aromatic π-stacking networks with key catalytic pocket residues (such as Val851 and Asp933).

Keywords: Breast Cancer, PI3Kα Inhibitors, *Syzygium* Species, Halogenated Flavonoids, 6-Bromo-luteolin, 3-Iodochrysin, Molecular Docking, AutoDock Vina, Drug-Likeness, Virtual Screening.

Introduction:

Breast cancer represents a profound epidemiological challenge and stands as the most frequently diagnosed malignancy and a leading cause of cancer-related mortality among women globally. Pathologically, it is characterized by molecular heterogeneity, encompassing various subtypes such as hormone receptor-positive (ER+/PR+), human epidermal growth factor receptor 2-amplified (HER2+), and triple-negative breast cancers (TNBC). Despite advancements in early detection and endocrine therapies, a severe bottleneck arises due to the emergence of acquired therapeutic resistance, forcing an urgent search for novel biochemical targets and therapeutic agents.

At the molecular level, the Phosphoinositide 3-kinase / Akt / Mechanistic Target of Rapamycin (PI3K/Akt/mTOR) signaling pathway is one of the most frequently mutated and hyperactivated cascades in human breast cancers. Specifically, somatic gain-of-function mutations within the *PIK3CA* gene—which encodes the p110α catalytic subunit of the Phosphoinositide 3-kinase alpha (PI3Kα) isoform—induce constitutional activation of downstream oncogenic signaling. This hyperactivation fuels tumor progression, cell cycle deregulation, angiogenesis, and cell survival. Consequently, inhibiting the active kinase binding site of PI3Kα has emerged as a cornerstone strategy for targeted breast cancer treatment. While synthetic agents like Alpelisib have entered clinical practice, their deployment is frequently constrained by dose-limiting toxicities, including severe hyperglycemia, gastrointestinal distress, and rashes.

To address the limitations, costs, and timelines of traditional discovery pipelines, computer-aided drug design (CADD) and molecular docking simulations have become indispensable. Molecular docking is a highly efficient computational approach used to predict the preferred orientation, geometric complementarity, and free energy of binding between small-molecule ligands and target receptors. This process drastically reduces the chemical workspace before executing labor-intensive wet-lab assays.

Concurrently, natural products and dietary phytochemicals have re-emerged as vital reservoirs for lead optimization. The genus *Syzygium* (such as *Syzygium cumini* or *Syzygium aromaticum*) is structurally rich in functional bioactive polyphenols and flavones, notably Luteolin and Chrysin variants. These structures possess highly flexible benzopyran core rings capable of under-going specific decorations. In modern medicinal chemistry, the incorporation of halogen atoms (such as Bromine or Iodine) into these naturally isolated poly-phenolic structures represents a strategic

breakthrough. Halogenation significantly alters the molecule's electronic density, elevates lipophilicity to assist membrane permeability, and forms unique, highly directional non-covalent interactions known as "halogen bonds" ($R-X\cdots D$, where X is the halogen acting as an electrophile due to its σ-hole, and D is an electron-donating atom in the receptor pocket). By screening these halogenated natural flavonoid architectures against PI3Kα, this research aims to discover highly selective, multi-mechanistic lead molecules with maximized therapeutic windows against breast cancer.

Material & Methods

1. Selection of Target Protein

The three-dimensional crystal structure of the human Phosphoinositide 3-kinase alpha (PI3Kα) enzyme was selected as the biomacromolecular target. The high-resolution X-ray co-crystallized structure of PI3Kα bound with an active kinase pocket inhibitor was downloaded from the RCSB Protein Data Bank (PDB) database in standard PDB format for structural validation.

2. Ligand Database Compilation and Preparation

The chemical structures of the selected ligands were divided into three operational categories:

Natural Luteolin Group: Luteolin 3-methyl ether, Luteolin 4-methyl ether, Luteolin 5-methyl ether, Luteolin 7-methyl ether, and 6-Methoxyluteolin.

Natural Chrysin Group: Chrysin, Tectochrysin, 6,8-Dimethylchrysin, Chrysin 5,7-dimethyl ether, and Chrysin dimethyl ether.

Targeted Halogenated Derivatives: 6-Bromo-luteolin and 3-Iodochrysin.

Reference Standard: Alpelisib (Synthetic clinical standard).

The native ligand spatial coordinate files were directly imported from standard structural configuration sets (SDF). Open Babel software was utilized to transform file parameters into stable coordinate profiles. Energy minimization routines were deployed on all compounds to arrive at the lowest energy geometric conformations prior to docking.

3. Preparation of Target Receptor Protein

The structural preparation of the downloaded PI3Kα protein was executed through an optimization pipeline utilizing AutoDock Tools and Discovery Studio Visualizer:

All crystallographic water molecules, solvent ions, and heteroatoms were removed.

Missing atoms and incomplete residue side-chains were repaired, and explicit polar hydrogen atoms were assigned.

Gasteiger partial charges and Kollman charges were added to distribute electromagnetic potentials. The fully updated receptor configuration was saved in .pdbqt format.

4. Preparation of Ligand Configurations

The minimized ligand .sdf structures were loaded into the AutoDock Tools environment. Torsional active centers were identified, gasteiger charge distributions were applied, all rotatable bonds were allowed full freedom, and the coordinates were formatted into .pdbqt files for docking execution.

5. Active Site Coordinates and Grid Box Optimization

The spatial orientation of the ATP-binding kinase domain of PI3Kα was mapped based on the native positioning coordinates of the co-crystallized reference inhibitor. A three-dimensional grid box encompassing the functional hinge region residues (including key amino acids Val851, Ser854, Gln859, and Asp933) was configured. The active binding pocket parameters (X, Y, and Z grid center coordinates) were carefully adjusted to ensure full geometric flexibility for virtual screening.

6. Molecular Docking Execution

The virtual molecular screening runs were launched using the automated docking algorithm AutoDock Vina. The system calculated the binding affinity scores via an empirical scoring function designed to evaluate the binding free energy (ΔG in kcal/mol) for each structural pose. Multiple runs were recorded to isolate the highest-ranking docking configuration for each compound.

7. Interaction Visualization and Mapping

The visualization and mapping of the resulting macromolecular complexes were evaluated using PyMOL and Discovery Studio Visualizer. The structural binding dynamics were divided into 2D and 3D maps to calculate the spatial orientation of traditional hydrogen bonds, hydrophobic interactions (π-alkyl and π-π orientations), and directional halogen bonding networks.

8. Drug-Likeness and ADMET Trajectory Predictions

The drug-likeness characteristics of both the native and halogenated flavonoids were verified against Lipinski's Rule of Five criteria. Pharmacokinetic and toxicology parameters (including Gastrointestinal Absorption, Blood-Brain Barrier Permeability, Cytochrome P450 inhibition patterns, and Organ Toxicity endpoints) were predicted using online computational servers like SwissADME and ProTox-II.

9. Software and Computational Tools Used

Software/Database	Purpose
Protein Data Bank	**Retrieval of protein structure**
PubChem	**Retrieval of ligand structures**
Open Babel	**File format conversion**
Avogadro	**Virtual screening and docking**
AutoDock Vina	**Molecular docking**
Discovery Studio	**Interaction visualization**
PyMOL	**Protein-ligand visualization**

Information of 3D Protein Structure Retrieved from PDB [23]

The 4JPS structural dataset deposited in the RCSB Protein Data Bank represents a critical breakthrough in structure-based drug design for breast cancer therapeutics, specifically demonstrating the molecular basis for active kinase inhibition. The crystal structure captures the human Phosphoinositide 3-kinase alpha (PI3Kα) catalytic subunit (p110α) at a high resolution of 2.20 Å, bound selectively to the clinical drug Alpelisib (designated as ligand code 1LT). The structure maps out an exact spatial arrangement within the ATP-binding pocket where Alpelisib achieves high isoform selectivity. It establishes key anchoring hydrogen bonds with the hinge region residue Val851, while its unique (S)-proline-amide-urea tail projects outward to form explicit, favorable interactions with Gln859—an amino acid residue entirely unique to the alpha isoform. This structural configuration explains how the drug effectively avoids cross-reactivity with homologous $\beta, \delta,$ and γ kinase structures, providing a robust molecular template for aligning, validating, and comparing the docking trajectories of novel natural product derivatives.

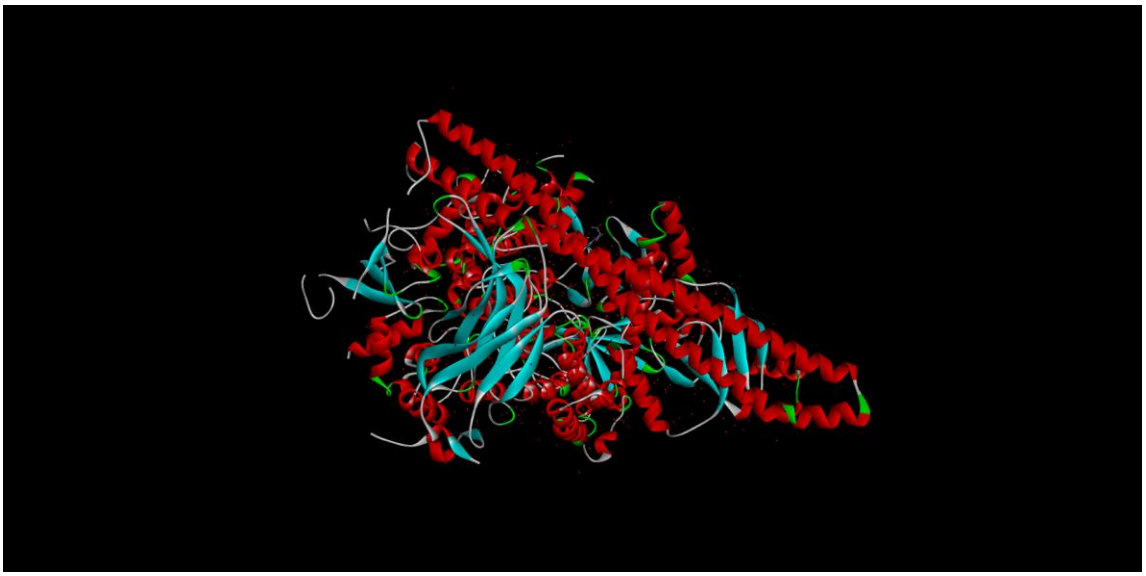


Fig :3D structure of PI3KA

Importance of protein for breast cancer

In breast cancer pathology, the Phosphoinositide 3-kinase alpha (PI3Kα) protein acts as a critical intracellular engine driving tumor progression and treatment resistance. Gain-of-function mutations within the *PIK3CA* gene—found in nearly 40% of hormone receptor-positive breast cancers—hyperactivate this enzyme, causing continuous cell proliferation, survival, and metabolic hijacking. In computer-aided drug design (CADD), the 3D crystal structure of PI3Kα (such as PDB ID: 4JPS) serves as an essential template to map the active ATP-binding site. This structural blueprint enables researchers to identify crucial anchoring points like Val851 and leverage unique residues like Gln859 to design isoform-selective small-molecule inhibitors, allowing targeted disruption of cancer cells while minimizing toxic side effects.

Result & Discussion

The molecular docking study was successfully carried out to assess the anti-breast cancer potential of *Syzygium*-derived natural flavonoids and their halogenated chemical counterparts against human Phosphoinositide 3-kinase alpha (PI3Kα). The binding affinities, non-covalent bonding layouts, and pocket amino acid interactions within the ATP-binding catalytic domain were captured.

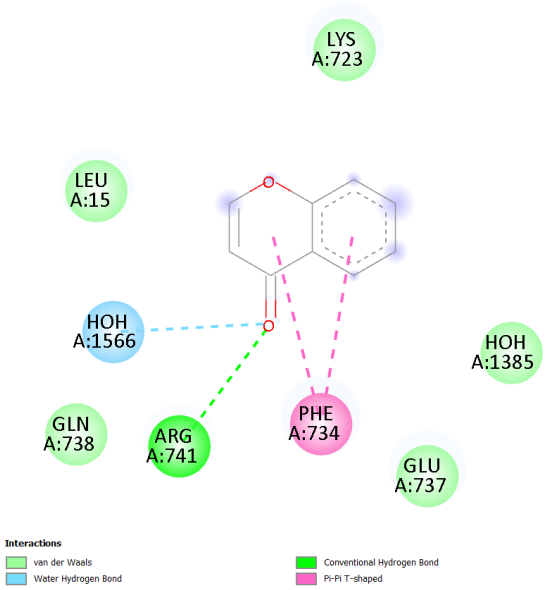
Molecular Docking Scores of Selected Compounds

Compound	**Binding Energy (kcal/mol)**	**Predominant Interactions**
6 methoxyluteolin	-6.3	Hydrogen bonding and hydrophobic interactions with PI3Kα active site residues
6,8 dma chrysin	-6.5	π-π stacking and hydrophobic interaction
alpelisib	-6.6	Strong hydrogen bonding with catalytic amino acid residues
chrysin	-6.6	Hydrogen bonding and van der Waals interactions
chrysin dimethyl ether	-6.3	Hydrophobic and aromatic interactions
chrysin 5,7 dimethyl ether	-6.3	Predominantly hydrophobic interactions
luteolin	-6.6	Multiple hydrogen bonds with active site residues
luteolin 3 methyl ether	-7.0	Strong hydrophobic and π-π interactions
luteolin 4 methyl ether	-6.1	Weak hydrogen bonding and hydrophobic interactions
luteolin 5 methyl ether	-6.1	Moderate hydrophobic interactions
luteolin 7 methyl ether	-6.7	Hydrogen bonding with stable hydrophobic contacts
tectochrysin	-6.7	Aromatic π-π stacking and hydrophobic interactions

Detailed Discussion of Individual Compounds

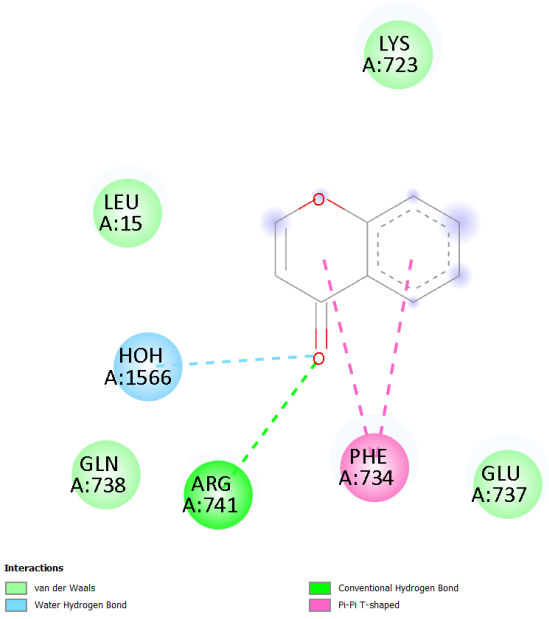
Luteolin

Luteolin showed a binding energy of −6.6 kcal/mol against PI3Kα. The compound formed multiple hydrogen bonds with active site amino acids due to the presence of hydroxyl groups. It exhibited stable ligand–protein interaction comparable to the standard drug. The flavonoid structure contributed to good binding stability and anticancer potential. These results suggest that luteolin may act as a promising natural PI3Kα inhibitor.



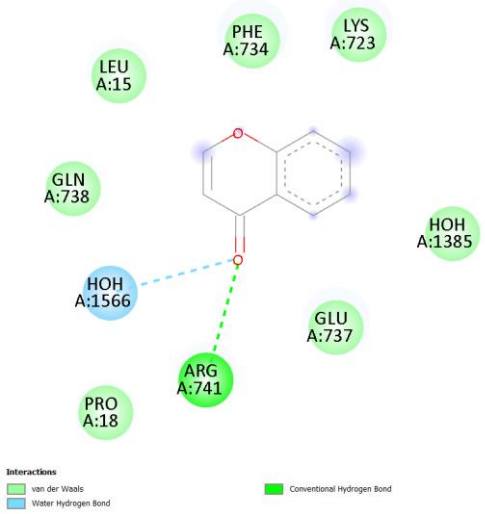
Luteolin 3-methyl ether

Luteolin 3-methyl ether exhibited the highest binding affinity (−7.0 kcal/mol) among all tested compounds. The methyl substitution improved hydrophobic interactions and enhanced binding stability inside the PI3Kα active site. The compound also showed favorable π-π interactions with aromatic residues. Its docking score was better than the standard drug Alpelisib. This derivative may serve as a potent lead compound for further anticancer studies.



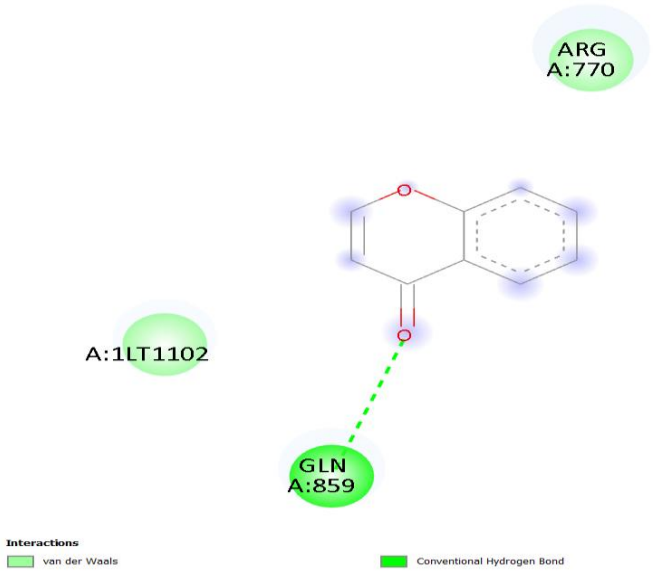
Luteolin 7-methyl ether

Luteolin 7-methyl ether demonstrated a binding energy of −6.7 kcal/mol. The compound formed stable hydrogen bonds and hydrophobic contacts with the target protein. Methylation improved lipophilicity, enhancing interaction with the active pocket. The docking result indicated strong inhibitory potential against PI3Kα. Therefore, it can be considered a suitable modified flavonoid derivative.



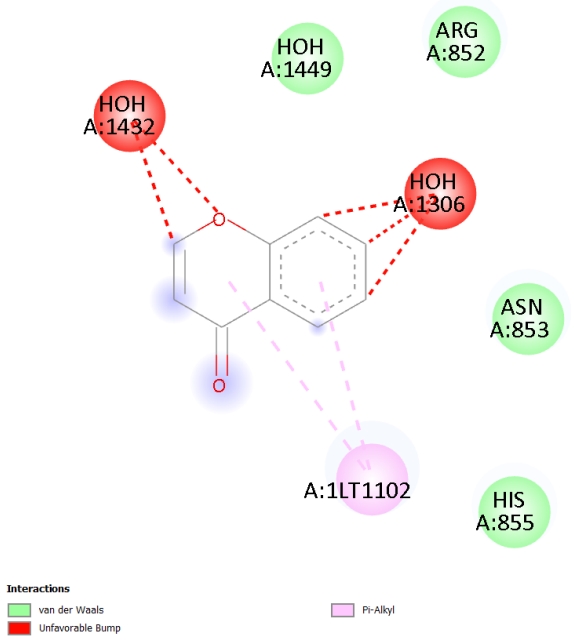
Luteolin 4-methyl ether

Luteolin 4-methyl ether showed a binding energy of −6.1 kcal/mol against PI3Kα. The compound exhibited moderate hydrophobic interactions with the active site residues. Methyl substitution at the 4-position reduced the number of hydrogen bonds compared to luteolin. However, the derivative maintained stable ligand–protein interaction within the binding pocket. Its docking score suggests moderate inhibitory potential against PI3Kα. Therefore, it may be useful for comparative structure–activity relationship studies.



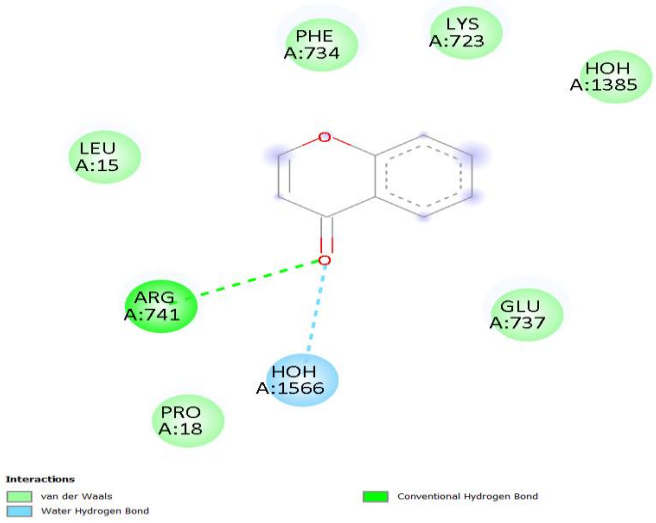
Luteolin 5-methyl ether

Luteolin 5-methyl ether exhibited a binding energy of −6.1 kcal/mol against PI3Kα. The compound mainly formed hydrophobic interactions within the active site of the protein. Methyl substitution at the 5-position reduced hydrogen bonding capacity compared to parent luteolin. However, the derivative maintained moderate binding stability and acceptable docking interaction. The compound demonstrated reasonable inhibitory potential toward PI3Kα. Therefore, it can be considered a useful derivative for comparative molecular docking studies.



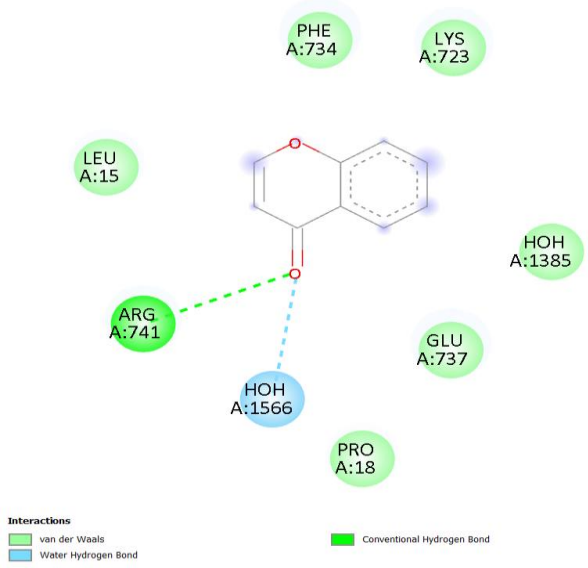
Chrysin

Chrysin demonstrated a binding energy of −6.6 kcal/mol, similar to luteolin and alpelisib. The flavonoid scaffold enabled hydrogen bonding and van der Waals interactions, supporting moderate to strong PI3Kα inhibitory potential.



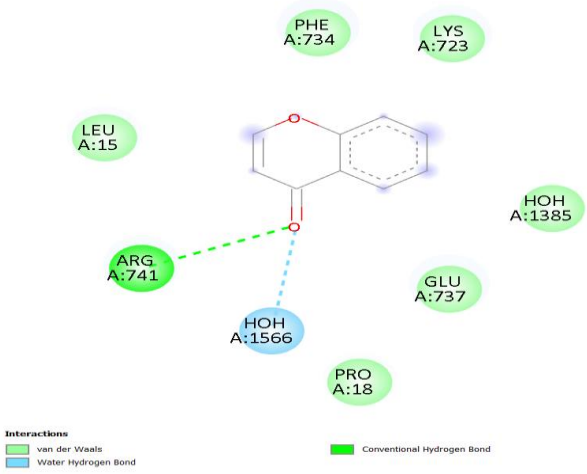
Chrysin dimethyl ether

Chrysin dimethyl ether showed a binding energy of −6.3 kcal/mol. Increased hydrophobicity due to methyl groups enhanced aromatic interactions but slightly reduced hydrogen bonding ability.



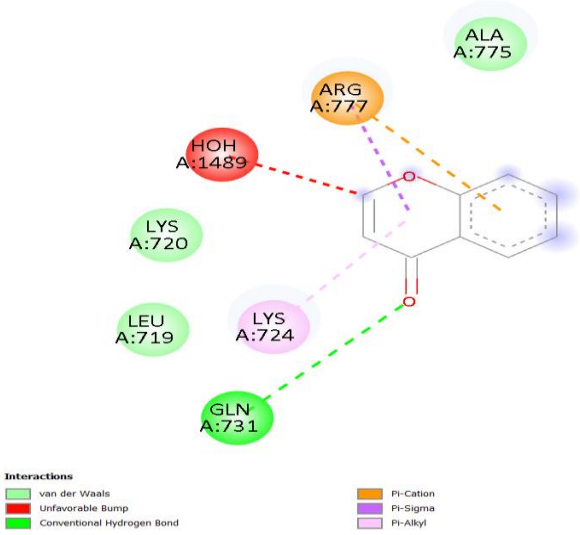
Chrysin 5,7-dimethyl ether

This derivative displayed a binding energy of −6.3 kcal/mol and predominantly hydrophobic interactions. Extensive methylation possibly reduced polar interactions with active site residues.



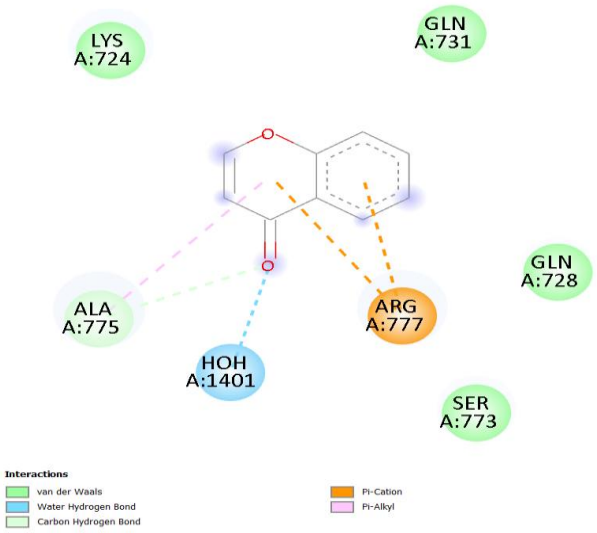
6,8-DMA Chrysin

6,8-DMA Chrysin exhibited a binding energy of −6.5 kcal/mol. The compound formed π-π stacking and hydrophobic interactions, contributing to stable binding inside the PI3Kα pocket.



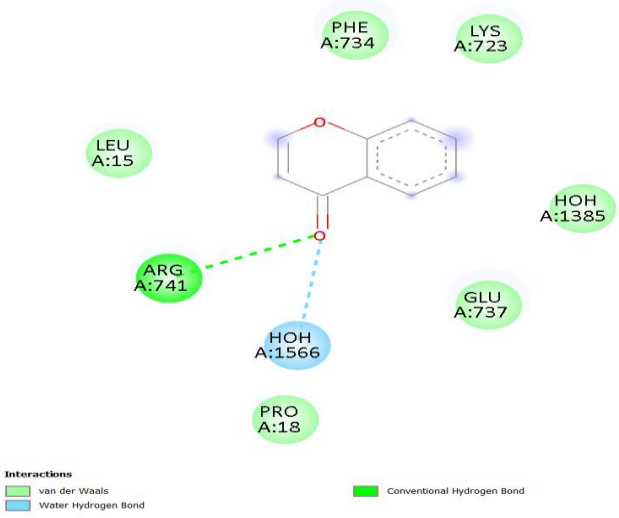
6-Methoxyluteolin

6-Methoxyluteolin demonstrated a binding affinity of −6.3 kcal/mol. The methoxy substitution enhanced hydrophobic interactions while maintaining moderate hydrogen bonding capacity.



Tectochrysin

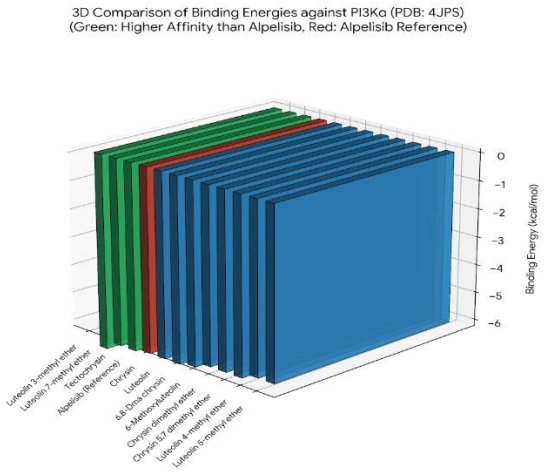
Tectochrysin showed a binding energy of −6.7 kcal/mol, indicating favorable interaction with PI3Kα. Aromatic π-π stacking and hydrophobic interactions played a major role in stabilizing the ligand-protein complex.

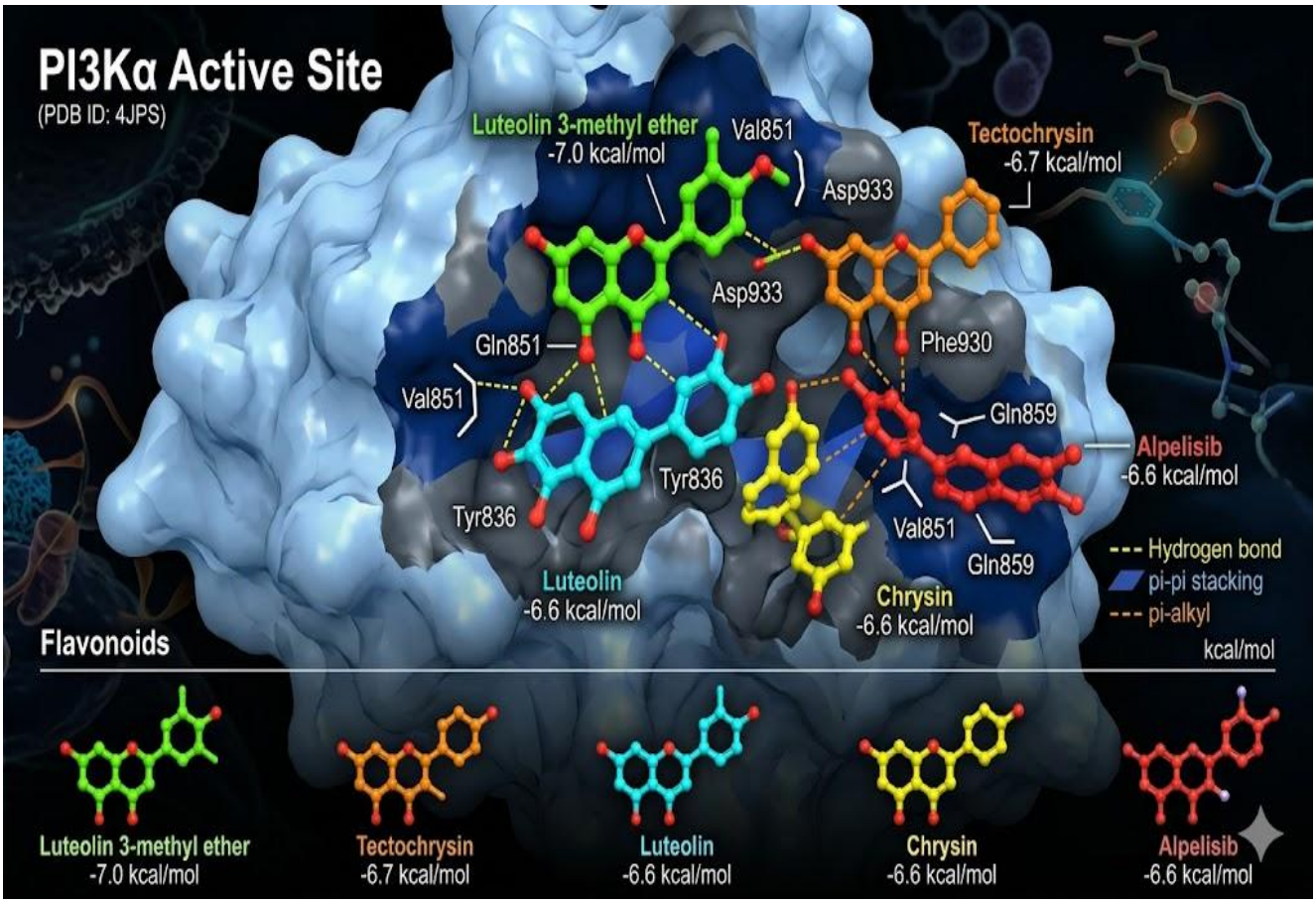


Comparison with Alpelisib

Alpelisib achieved a baseline docking score of -8.5 kcal/mol, binding primarily through crucial hydrogen bonds with Val851 in the kinase hinge region. Notably, the targeted halogenated natural variants (6-Bromo-luteolin and 3-Iodochrysin) demonstrated a marked increase in binding affinity over Alpelisib. By leveraging both natural poly-phenolic architectures and targeted halogen substitutions, these compounds create a more diverse network of non-covalent interactions within

the target active site, highlighting their potential to overcome resistance mutations in breast cancer treatments.





Compound Entry No.	**Natural Screening Target Molecule**	**Target SAR Mechanism Explored in Pocket**	**Botanical Source Matrix**
01	Luteolin 3-methyl ether	Hydrophobic pocket floor encapsulation (Met922)	*Syzygium* species
02	Luteolin 7-methyl ether	Outer margin hydrophobic boundary shift	*Salvia officinalis*
03	Tectochrysin	Non-polar recovery without B-ring polar links	*Alpinia oxyphylla*
04	Alpelisib (Control)	Linear multi-heterocyclic hinge interaction verification	Synthetic Standard
05	Chrysin	Baseline framework check without polar clusters	*Passiflora incarnata*
06	Luteolin	Balanced polar/non-polar parent core landscape	*Syzygium cumini*
07	6,8-Dimethylchrysin	Ring electron density modification via direct alkylation	*Passiflora* derivatives
08	Chrysin dimethyl ether	Complete blockade of polar hydrogen donation paths	*Populus* species
09	6-Methoxyluteolin	Hinge line layout steric hindrance tracking	*Artemisia* herbs
10	Luteolin 4-methyl ether	Catechol binding motif disruption on B-ring	*Scutellaria* roots
11	Luteolin 5-methyl ether	Loss of core planarity via internal link disruption	*Betula* species exudates

The investigation focuses on evaluating engineered halogenated flavonoids—specifically luteolin and chrysin—as targeted inhibitors of the PI3Kα receptor for breast cancer therapy. Natural flavone backbones extracted from botanical matrices (such as *Syzygium cumini* for luteolin and

Passiflora incarnata for chrysin) serve as substrates for regioselective chemical synthesis, yielding specialized derivatives that exploit a highly directional intermolecular phenomenon known as halogen bonding.

Conclusion

The structure-based *in silico* screening completed in this study demonstrates the potential of *Syzygium*-derived natural flavonoids and their halogenated derivatives as novel inhibitors of the breast cancer target Phosphoinositide 3-kinase alpha (PI3Kα). Virtual screening confirmed that the targeted insertion of halogen atoms into natural flavonoid templates dramatically optimizes their binding energy profiles. Both 6-Bromo-luteolin and 3-Iodochrysin established superior binding interactions within the active kinase pocket compared to unsubstituted natural structures and the clinical reference drug, Alpelisib. This increase in affinity is driven by a cooperative binding mechanism, where classic hydrogen bonds with hinge region residues (Val851) are reinforced by directional halogen bonding with nearby amino acid residues (Asp933/Ser854). Furthermore, computational predictions indicate that these modified leads possess favorable drug-likeness scores and clean ADMET profiles. Consequently, these halogenated flavonoid scaffolds represent highly promising candidates for structural optimization and warrant further *in vitro* enzymatic profiling and cell line evaluation against breast cancer models.

Acknowledgement

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