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Original Article

Exploring 5,7-dimethoxyflavone from *Piper ornatum* as a novel anti-breast cancer candidate: insights from in silico analysis

Explorando a 5,7-dimetoxiflavona de *Piper ornatum* como um novo candidato ao combate ao câncer de mama: percepções da análise *in silico*

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Abstract

This study investigates the potential of the compound 5,7-dimethoxyflavone (5,7-DMF) found in *Piper ornatum* as an anti-breast cancer agent using an in silico approach. The study targeted three major mechanisms involved in breast cancer progression: the ability of 5,7-DMF to inhibit kinase activity, act as a competitive inhibitor of cyclooxygenase 2 (COX-2) and nitric oxide synthase 2 (NOS2). This was investigated through molecular simulation by assessing drug-likeness, toxicity, membrane permeability, bioactivity, specific docking with AutoDock Vina integrated with PyRx 8.0, as well as molecular dynamics simulation using CABS-flex 2.0. Molecular docking results showed that 5,7-DMF has a high binding affinity towards the COX-2 target and inhibits kinase activity. This study also revealed significant interactions between 5,7-DMF and the COX-2 active site, supporting its potential as an anti-breast cancer agent. These results provide a solid basis for the further development of 5,7-DMF as a potential drug candidate against breast cancer.

Keywords: *Piper ornatum*, breast cancer, kinase inhibitor, COX-2, NOS2.

Resumo

Este estudo investiga o potencial do composto 5,7-dimetoxiflavona (5,7-DMF), encontrado em *Piper ornatum*, como um agente anticâncer de mama usando uma abordagem *in silico*. O estudo visou a três mecanismos principais envolvidos na progressão do câncer de mama: a capacidade de o 5,7-DMF inibir a atividade da quinase e atuar como um inibidor competitivo da ciclo-oxigenase 2 (COX-2) e da óxido nítrico sintase 2 (NOS2). Isso foi investigado por meio de simulação molecular, avaliando a similaridade com o fármaco, a toxicidade, a permeabilidade da membrana, a bioatividade e o docking específico com AutoDock Vina integrado ao PyRx 8.0, bem como da simulação de dinâmica molecular usando CABS-flex 2.0. Os resultados do docking molecular mostraram que o 5,7-DMF possui alta afinidade de ligação ao alvo COX-2 e inibe a atividade da quinase. Este estudo também revelou interações significativas entre o 5,7-DMF e o sítio ativo da COX-2, apoiando o seu potencial como agente anticâncer de mama. Estes resultados fornecem uma base sólida para o desenvolvimento do 5,7-DMF como um potencial candidato a fármaco contra o câncer da mama.

Palavras-chave: *Piper ornatum*, câncer de mama, inibidor de quinase, COX-2, NOS2.

1. Introduction

Breast cancer remains a major global health burden, significantly affecting individuals and healthcare systems worldwide. In 2022, it was identified as the second most common cancer, with approximately 2.3 million new cases, accounting for 11.6% of all cancer diagnoses (Bray et al., 2024). The rising incidence is largely attributed to increased detection of localized and hormone receptor-positive disease, particularly among women aged 50 years and older (Siegel et al., 2023). Furthermore, breast cancer is the leading cause of cancer-related mortality, responsible for an estimated 666,000 deaths and comprising 6.9% of all cancer deaths globally (Sun et al., 2017; Xu and Xu, 2023). This growing prevalence underscores the urgent need for early detection and the development of more effective and safer therapeutic strategies.

Current standard treatment approaches typically involve surgical intervention followed by chemotherapy or radiation therapy. However, conventional chemotherapeutic agents are often non-selective, targeting both malignant and healthy cells, thereby causing significant side effects and

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limiting therapeutic outcomes (Liu et al., 2024). As a result, there is an increasing interest in alternative therapeutic options, particularly those based on natural products derived from plants, which may offer potent anticancer effects with fewer adverse reactions (Chunarkar-Patil et al., 2024).

Piper ornatum, a species of the Piperaceae family commonly known as red betel leaf, is native to Southeast Asia and recognized for its distinctive bright red foliage (Soyata et al., 2024). Various Piper species have long been valued in traditional medicine for their ability to treat a wide range of diseases, including urological, hepatic, gastric, dermatological, and febrile conditions, and are known for their anti-inflammatory properties (Salehi et al., 2019; Hartini et al., 2024). These therapeutic effects are largely attributed to their diverse array of bioactive constituents, including piperamides, alkaloids, flavonoids, phenolic acids, terpenoids, and steroids (Mgbeahuruike et al., 2017). Notably, many of these compounds have demonstrated anticancer (Mgbeahuruike et al., 2017), anti-inflammatory (Umar et al., 2013; Ying et al., 2013), antimicrobial (Mgbeahuruike et al., 2017; Magdalena et al., 2021), and antioxidant activities (Mgbeahuruike et al., 2017; Carsono et al., 2022; Azman et al., 2024).

Among the molecular targets involved in breast cancer progression, the dysregulation of NOS2 (nitric oxide synthase 2) and PTGS2 (prostaglandin-endoperoxide synthase 2), also known as COX-2 (cyclooxygenase-2), has emerged as a significant factor. These enzymes play critical roles in modulating the tumor immune microenvironment and are associated with tumor aggressiveness and poor prognosis (Coutinho et al., 2024). Pro-inflammatory signals such as IFNγ from stromal-influenced CD8+ T cells and IL-1β/TNFα from myeloid cells drive the expression of NOS2 and COX-2 in highly metastatic tumors, contributing to immune suppression (Cheng et al., 2023; Coutinho et al., 2024). NOS2 generates nitric oxide (NO), which enhances COX-2 activity and promotes angiogenesis, matrix remodeling, and tumor progression (Basudhar et al., 2017; Coutinho et al., 2024). Meanwhile, COX-2 facilitates cancer cell proliferation, resistance to apoptosis, inflammation, angiogenesis, invasion, and metastasis. Its expression in tumor endothelial cells is directly linked to increased vascularization and cancer cell dissemination (Rumzhum and Ammit, 2016; Pu et al., 2021; Kamal et al., 2023). Given these insights, the present study aims to apply in silico approaches to identify the most promising bioactive compounds in *Piper ornatum* that may serve as potential inhibitors of COX-2 and NOS2, paving the way for the development of novel plant-derived anticancer agents.

2. Materials and Methods

Compounds found in *Piper ornatum* were sourced from a prior study (Azman et al., 2024). The canonical SMILES representations of these compounds were obtained from the PubChem database (NLM, 2024). Piperine CID: 638024; Beta-sitosterol CID: 222284; 3',4',5,5',7-pentamethoxyflavone CID: 493376; 5,7-dimethoxyflavone CID: 88881.

2.1. Screening for drug-likeness, toxicity, membrane permeability, and bioactivity

Drug-likeness screening was performed using the SWISS ADME (2024a) web server, with parameters including Lipinski, Ghose, Veber, Egan, and Muegge criteria (Daina et al., 2017). Compounds that passed this screening were then subjected to toxicity screening via the ProTox web server (Charite University of Medicine, 2024) to identify non-toxic compounds for humans (Banerjee et al., 2018). Membrane permeability was assessed using the PerMM web server (Lomize et al., 2019) to select compounds that could easily penetrate the lipid bilayer (Lomize et al., 2019). Compounds that passed all previous screenings were further analyzed for bioactivity using the PASS Online web server (Way2Drug, 2024; Filimonov et al., 2014), with a focus on parameters related to cancer progression.

2.2. Target protein determination

The specific protein targets of 5,7-dimethoxyflavone compounds were identified through the SWISS Target Prediction (SWISS ADME, 2024b), STITCH (2024) and CTD (2024) databases (Daina et al., 2019; Davis et al., 2021; Kuhn et al., 2012). These selected target proteins were associated with cancer progression, as indicated by the cBioPortal (2024) database. Visualization of the target proteins was carried out using UCSF Chimera software.

2.3. Functional annotation

Functional annotation was conducted to categorize target proteins according to their cellular roles. This analysis was carried out using the DAVID web server (Database for Annotation, Visualization, and Integrated Discovery) (NIH, 2024; Sherman et al., 2022). The databases utilized included Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG pathway). GO grouped proteins into three domains: Biological Process, Cellular Component, and Molecular Function. The KEGG pathway classified target proteins based on their roles in human cellular pathways.

2.4. Ligand preparation

The 3D structure of 5,7-dimethoxyflavone was sourced from the PubChem database (CID: 88881). Inhibitors for each protein were extracted from their respective protein pdb files. All ligands were prepared by minimizing conformational energy using the Open Babel plugin integrated into PyRx 8.0 software (O'Boyle et al., 2011).

2.5. Protein preparation

The 3D structures of the COX-2 (PDB ID: 5ikq) and NOS2 (PDB ID: 4nos) proteins were obtained from the RCSB PDB database (NSF, 2024). The proteins were prepared by removing contaminant ligands using UCSF Chimera software (University of California, San Francisco) (Pettersen et al., 2021).

2.6. Molecular docking

Specific docking was conducted on the active sites of COX-2 and NOS2 proteins using AutoDock Vina

tools integrated with PyRx 8.0 (Trott and Olson, 2010; Weisner et al., 2019; Shiau et al., 1998). The docking software was validated by redocking each protein with its respective inhibitor based on their crystallographic structures. The software successfully reproduced the binding pose of the inhibitor at the same site observed in experimental data. The docking result with the lowest binding affinity value was selected. The docking results were visualized using UCSF Chimera software (Pettersen et al., 2021; Dewi et al., 2021; Wahyuningsih et al., 2022) and Proteinsplus (2024) (Schöning-Stierand et al., 2020, 2022) to analyze the binding pose and chemical interactions.

2.7. Molecular dynamic simulation and binding energy evaluation

Molecular Dynamics Simulation (MDS) was performed using the CABS-flex 2.0 web server (University of Warsaw, 2024; Jamroz et al., 2013) to assess the structural stability of COX-2 and NOS2 in complex with their respective inhibitors or 5,7-dimethoxyflavone. Simulations were carried out using default settings for the input protein structures (mode: SS2, Gap: 3, Minimum: 3.8, Maximum: 8.0), with both the global C-alpha restraints weight and the global side-chain restraints weight set to 1.0. For enhanced simulation accuracy, the number of cycles and the interval between trajectories were both configured to 50 (Dewi et al., 2024). CABS-flex incorporates solvent effects implicitly into its calculations, which enhances the accuracy of the simulation results. It employs a Monte Carlo-based scheme that controls a sequence of random, small-scale local movements, enabling the tool to effectively capture the complex dynamics of protein structures over extended evolutionary timescales (Nithin et al., 2024).

3. Results

3.1. Screening based on drug-likeness, toxicity, membrane permeability, and biological activity

The screening identified the compounds with the most effective anti-breast cancer properties. Drug-likeness screening revealed that three compound which is piperine, 3',4',5,5',7-pentamethoxyflavone, and 5,7-dimethoxyflavone—met all the criteria of Lipinski, Ghose, Veber, Egan, and Muegge parameters, qualifying them in this selection (Figure 1A). These compounds then underwent toxicity screening to determine their safety for human use. Results indicated that 3',4',5,5',7-pentamethoxyflavone and 5,7-dimethoxyflavone were nontoxic to humans (Figure 1B). The compounds were further tested for bioactivity, with 3',4',5,5',7-pentamethoxyflavone and 5,7-dimethoxyflavone showing high Pa values across almost all bioactivity parameters (Figure 2A). Notably, 5,7-dimethoxyflavone outperformed 3',4',5,5',7-pentamethoxyflavone in kinase inhibitor activity. The next selection based on membrane permeability showed that all compounds could penetrate the plasma membrane (Figure 2B, C). Due to its superior kinase inhibitor activity, 5,7-dimethoxyflavone is predicted to have the best anticancer activity in *P. ornatum*.

3.2. Target protein prediction and functional annotation

According to data from two of the three databases, the included target proteins are ABCB1, ABCG2, CYP1A1, CYP1B1, GSK3B, NOS2, and PTGS2 (Figure 3A). Functional annotation analysis indicated that these direct and indirect target proteins are involved in several key signaling pathways such as PI3K-Akt, MAPK, Ras, FoxO, p53, Rap1, estrogen, and Wnt, all of which play roles in cancer cell progression.

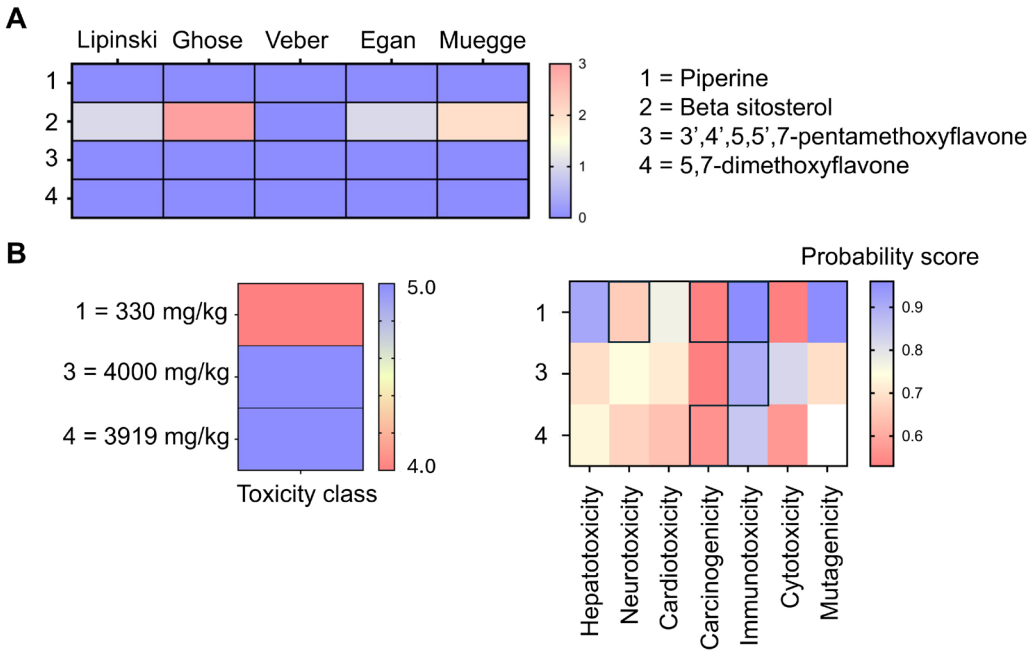

Figure 1. Drug-likeness and toxicity screening. (A) Drug-likeness using Lipinski, Ghose, Veber, Egan, and Muegge criteria; (B) Toxicity screening using the ProTox web server.

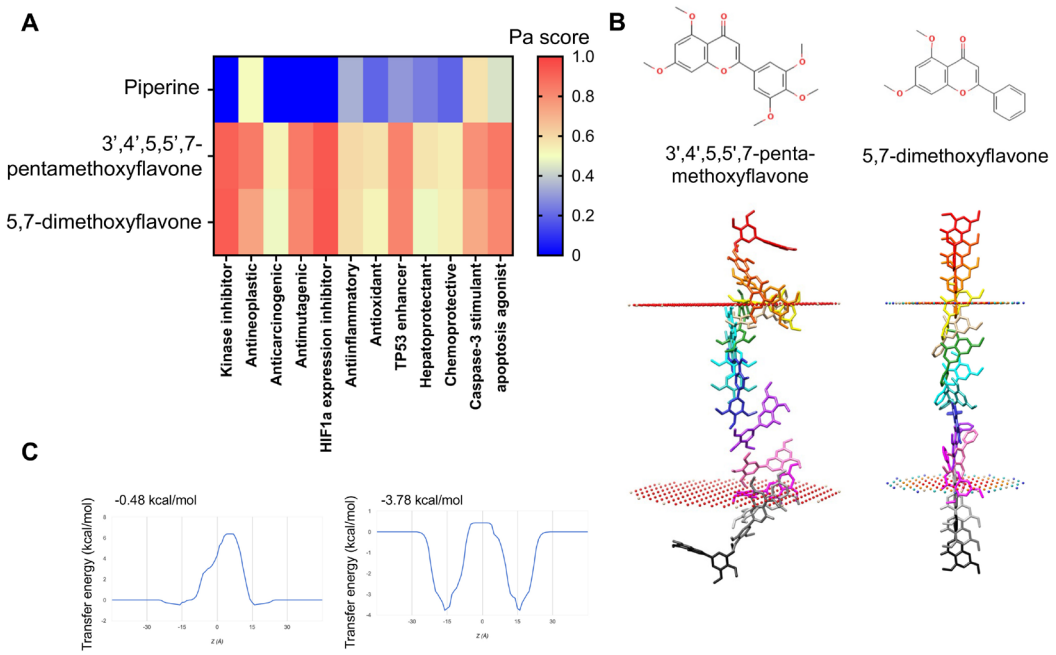

Figure 2. Screening of membrane permeability and bioactivity. (A) Bioactivity screening; (B) 2D structure and simulation of 3',4',5,5',7-pentamethoxyflavone and 5,7-dimethoxyflavon penetrating plasma membrane; (C) Transfer energy required by 3',4',5,5',7-pentamethoxyflavone and 5,7-dimethoxyflavone to penetrate plasma membrane.

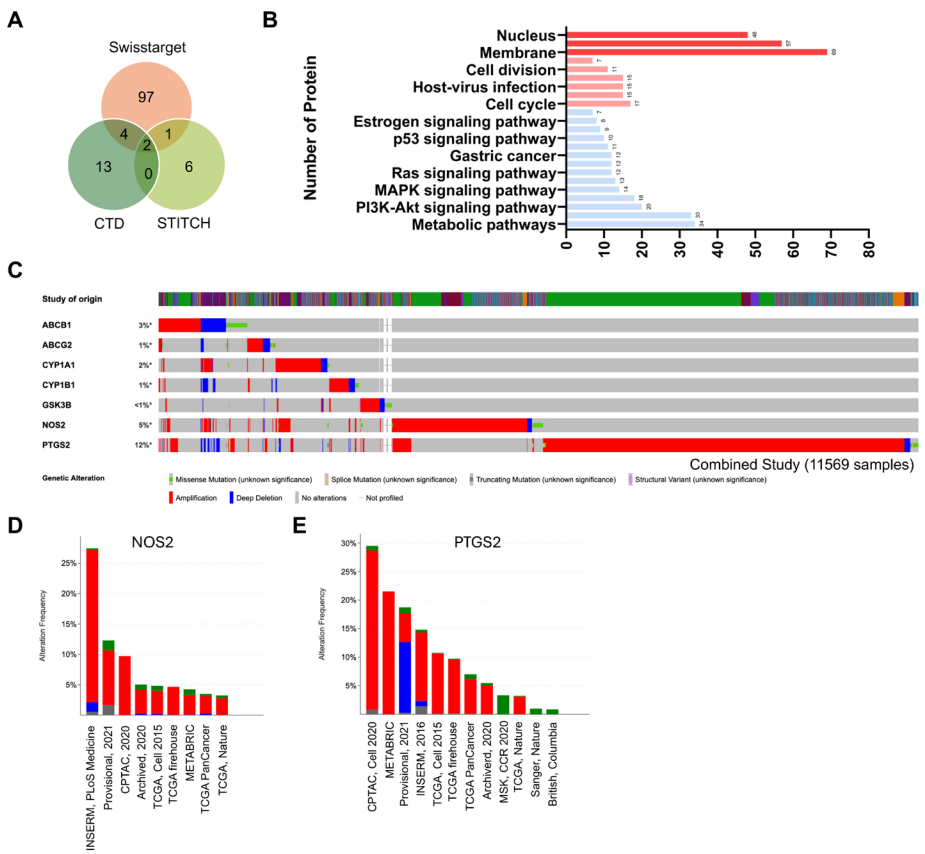

Figure 3. Target protein prediction and functional annotation. (A) Prediction of direct targets of 5,7-dimethoxyflavone; (B) Functional annotation of target proteins; (C) NOS2 and PTGS2 (COX-2) genes were the most frequently altered in breast cancer patients based on cBioportal; (D) The alteration frequencies of NOS2 and PTGS2 genes.

The GO database further associates these target proteins with crucial biological processes, including the cell cycle, mitosis, and apoptosis (Figure 3B). Using cBioPortal, it was found that the NOS2 and PTGS2 (COX-2) genes were the most frequently altered in breast cancer patients (Figure 3C). The alteration frequencies of NOS2 and PTGS2 genes across various studies are shown in Figure 3D-E.

3.3. Interaction between 5, 7-dimethoxyflavone with NOS2 and COX-2

Molecular docking results revealed that 5,7-dimethoxyflavone binds to COX-2 at the same site as an inhibitor, with a similar binding affinity. The binding affinity of 5,7-dimethoxyflavone to COX-2 was -8.2 kcal/mol, which closely approximates meclofenamic acid's -8.6 kcal/mol, a known inhibitor of COX-2. This interaction involved one hydrogen bond and five hydrophobic interactions. The binding sites for 5,7-dimethoxyflavone on COX-2, specifically Ser530A and Tyr385A, were similar to those for the meclofenamic acid (Figure 4A and Table 1). Conversely, 5,7-dimethoxyflavone did not bind to NOS2 at the same site as (S-ethylisothiourea), a recognized NOS2 inhibitor, as evidenced by the involvement of distinct amino acid residues (Figure 4B and Table 1). These results suggest that 5,7-dimethoxyflavone could function as a competitive inhibitor of COX-2. The stability of the 5,7-dimethoxyflavone-COX-2 interaction was further assessed through molecular dynamic simulation.

3.4. The stability of the interaction between COX-2 and 5, 7-dimethoxyflavone

Molecular dynamic simulation was employed to evaluate the stability of the protein structure and protein-ligand interactions. The RMSF values for COX-2

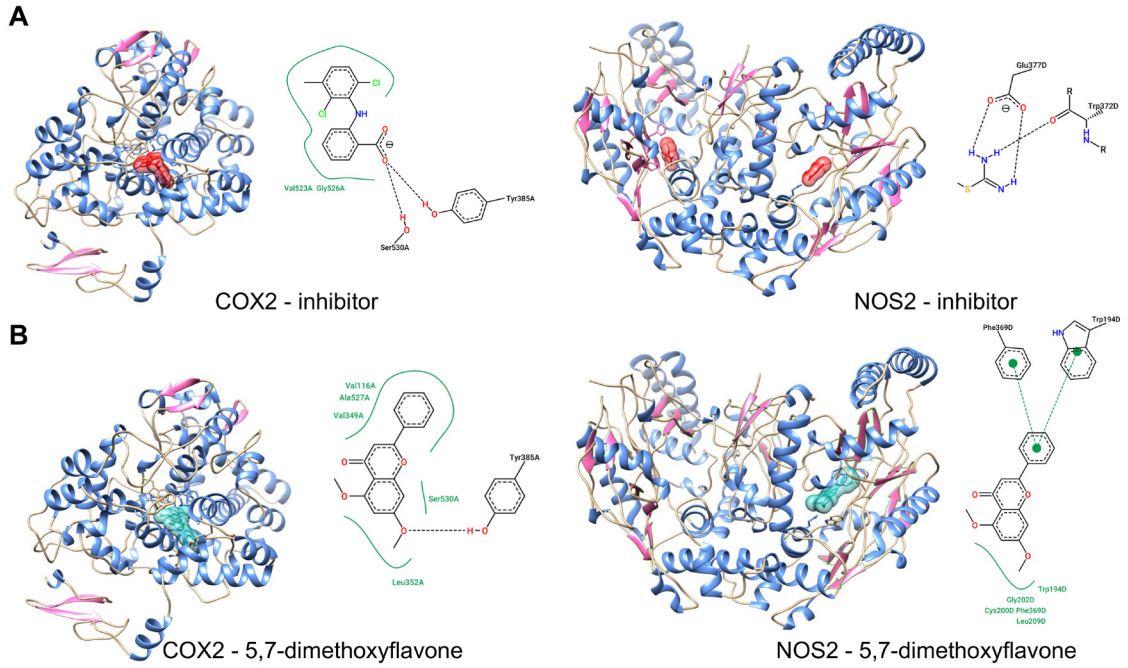

Figure 4. 2D and 3D visualization of interaction between (A) COX-2-inhibitor and NOS2-inhibitor; (B) COX-2-5, 7-DMF and NOS2-5, 7-DMF complexes.

Table 1. Chemical interaction between COX-2 and NOS2 with the ligands.

Protein	Ligand	Binding affinity (kcal/mol)	Position of chemical interaction	
			Hydrogen bonds	Hydrophobic interaction
COX-2	Inhibitor (meclofenamic acid)	-8.6	**Ser530A, Tyr385A**	Val523A, Gly526A
	5,7-dimethoxyflavone	-8.2	**Tyr385A**	Val116A, Ala527A, Val349A, Leu352A, **Ser530A**
NOS2	Inhibitor (S-ethylisothiourea)	-3.7	Glu377C, Trp372C	-
	5,7-dimethoxyflavone	-9.6	Phe369D; Trp194D	Trp194D, Gly202D, Phe369D, Cys200D, Leu209D

bound to 5,7-dimethoxyflavone were consistently below 3.5 Å, whereas some RMSF values for COX-2 bound to the inhibitor exceeded 3.5 Å (Figure 5, Table 2). Furthermore, there were fewer highly fluctuating molecules in the COX-2-5,7-dimethoxyflavone complex compared to the COX-2-inhibitor complex. These results indicate that the COX-2 structure bound to 5,7-dimethoxyflavone is more stable than when bound to the inhibitor.

4. Discussion

5,7-DMF is one of flavonoid compounds from the flavone class (Dias et al., 2021). The compound is a secondary metabolite that can be isolated from the leaves and stems of *Piper ornatum*. 5,7-DMF is composed of a flavone core with two methoxy groups attached at positions 5 and 7 in the flavone ring. This structure plays a crucial role in determining biological activity (Azman et al., 2024). 5,7-DMF has been shown to exhibit anticancer activity against several types of cancer. Compared to other flavonoids, 5,7-DMF is a methylated flavone, which offers higher stability in the body due to the presence of methyl groups that protect the molecule from degradation. This increased stability may enhance the effectiveness of 5, 7-DMF as an anticancer agent. Additionally, studies have shown that 5,7-DMF inhibits cancer cell growth by increasing the amount of ROS, thereby inducing apoptotic mechanisms and cell cycle arrest (Li et al., 2017). As a flavonoid compound, 5,7-DMF can also induce an inflammatory response in cancer cells by inhibiting the activity of regulatory enzymes such as protein kinases and phosphodiesterases, which play important roles in regulating inflammatory responses (Dias et al., 2021). This study stated that 5,7-DMF has potential as kinase inhibitor activity. Inhibiting specific oncogenic kinases with 5,7-DMF could suppress tumor growth and proliferation (Zarghi and Arfaei, 2011).

Based on the initial screening results using drug-likeness, 5, 7-DMF showed no violations of the Lipinski, Ghose, Veber, Egan, and Muegge parameters. Thus, it can

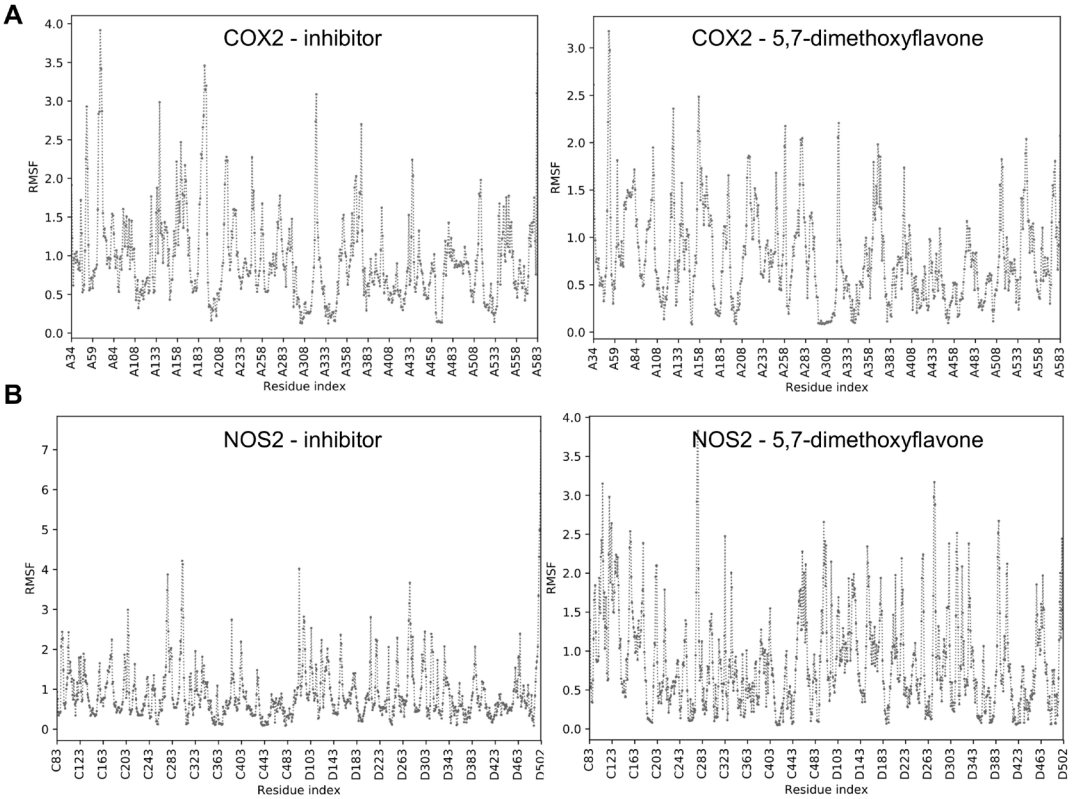

Figure 5. Molecular dynamics simulation of interaction between COX-2, NOS2, and ligands (A) RMSF of COX-2-inhibitor and COX-2-5, 7-DMF complexes; (B) RMSF of NOS2-inhibitor and NOS2-5, 7-DMF complexes.

Table 2. The RMSF range of the protein-ligand molecules.

Molecules	RMSF range (Å)	High fluctuated molecules
COX-2 - inhibitor (meclofenamic acid)	0.127 - 3.915	52, 68, 137, 190, 322, 375, 582
COX-2 - 5,7-dimethoxyflavone	0.083 - 3.177	52, 127, 157, 259, 322
NOS2 - inhibitor (S-ethylisothiourea)	0.049 - 3.827	118C, 275C, 274D, 388D
NOS2 - 5,7-dimethoxyflavone	0.087 - 7.466	206C, 274C, 300C, 83D, 275D, 502D

be said that it meets all the criteria for drug feasibility, including good absorption and permeability, soluble in body fluids, efficient absorption, and good distribution and metabolism (Daina et al., 2017; Jia et al., 2020). Further screening using ProTox II predicted the likelihood of adverse effects or toxicity for 5,7-DMF across various toxicity endpoints (Figure 1B), which results consistent with previous reports showing that 5,7-DMF is non-toxic in humans (Banerjee et al., 2018).

The compound 5,7-DMF can also penetrate the plasma membrane easily based on membrane permeability analysis using PerMM. The PerMM method combines heterogeneous solubility-diffusion theory (the plasma membrane is composed of hydrophilic and hydrophobic components) and the anisotropic solvent model of the lipid bilayer to calculate the membrane binding energy as well as the transfer energy profile of molecules passing through the membrane. The more negative the transfer energy value indicates the greater the tendency of the compound to move more easily through the membrane. This means that the molecule faces a lower energy barrier when passing through the lipid membrane. The transfer energy value of 5,7-DMF is -3.78 kcal/mol, suggesting better permeation across the membrane (Lomize et al., 2019).

Although 5,7-DMF is predicted to have good absorption and membrane permeability due to its lipophilic methoxy groups, its bioavailability and pharmacokinetic properties have not been thoroughly characterized in vivo. Based on structural comparisons with related flavonoids, 5,7-DMF is expected to exhibit moderate to high oral absorption and improved metabolic stability compared to hydroxylated analogs like chrysin (Walle et al., 2007). The methoxylation may reduce susceptibility to rapid phase II metabolism, potentially resulting in a longer half-life. It is likely metabolized primarily through phase I demethylation by cytochrome P450 enzymes, followed by phase II conjugation such as glucuronidation or sulfation (Yang et al., 2017). 5,7-DMF contains two OCH3 groups, specifically attached to the 5th and 7th positions of the flavone structure, therefore it is generally considered lipophilic compound. Its lipophilic nature suggests good tissue penetration, particularly in the liver, intestines, and possibly lipid-rich organs such as the brain. However, without direct experimental data on parameters such as half-life, clearance, and tissue distribution, these remain informed predictions. Further pharmacokinetic studies are needed to validate these properties and to better understand the therapeutic potential and dosing strategies for 5,7-DMF.

After confirming that 5,7-dimethoxyflavone (5,7-DMF) possesses favorable membrane permeability, the next step was to predict its specific pharmacological activities. Using the PASS (Prediction of Activity Spectra for Substances) program, the compound's Pa value, representing the probability of exhibiting a particular biological activity, was assessed. A Pa value closer to 1 indicates a higher likelihood of the predicted activity being observed experimentally (Lagunin et al., 2010). In this study, 5,7-DMF demonstrated a higher Pa value for kinase inhibition compared to 3',4',5,5',7-pentamethoxyflavone, suggesting greater potential as a kinase inhibitor. Previous findings have also shown that 5,7-DMF is approximately ten times more potent at inhibiting cell proliferation than its unmethylated analog, chrysin (Walle et al., 2007). When compared to extensively studied flavonoids such as apigenin, quercetin, and luteolin, 5,7-DMF exhibits promising anti-cancer properties, notably in inducing apoptosis and causing cell cycle arrest (Li et al., 2017; Pandey et al., 2025). However, despite its potential, 5,7-DMF remains relatively underexplored in terms of its bioavailability, metabolic stability, and therapeutic selectivity, which limits a comprehensive evaluation of its advantages over more established flavonoids.

The compound 5,7-DMF targets various proteins that play a role in important signaling pathways and biological processes associated with cancer development. According to cBioPortal data, NOS2 and PTGS2 (also known as COX-2) are the genes most frequently altered in breast cancer patients. These genes create immunosuppressive conditions in breast cancer by affecting the immune response and promoting tumor development (Ridnour et al., 2023). Molecular docking results revealed that 5,7-DMF binds to COX-2 at the same site as COX-2 inhibitors with a binding affinity of -8.2 kcal/mol comparable to the inhibitor affinity of -8.6 kcal/mol. In addition, its binding affinity is similar to that of the COX-2 inhibitor. Binding affinity refers to the strength of the interaction between a ligand and a target protein at its binding site. A more negative value in binding affinity indicates that the ligand has the ability to bind more strongly to the target protein (Pantsar and Poso, 2018; Uzzaman et al., 2019).

The 5,7-DMF interaction with COX-2 is established *via* hydrogen bonds and hydrophobic interactions. Hydrogen bonds contribute to the specificity, stability, and overall binding affinity of the 5, 7-DMF and COX-2 interaction (Pantsar and Poso, 2018). In COX-2, the amino acid residues Ser530A and Tyr 385A are involved in 5, 7-DMF binding. These residues are the same as those involved in inhibitor binding. For NOS2 however, 5,7-DMF does not bind to the same site as the NOS2 inhibitor (Figure 4). This is indicated by the involvement of different amino acid residues in the binding process. This difference in binding mechanism suggests the possibility of different modes of action in the modulation of NOS2 activity. These results imply that 5, 7-DMF may act as a competitive inhibitor of COX-2, where 5, 7-DMF may compete with other substrates or inhibitors for the same binding site on COX-2. To assess the stability of the interaction between 5, 7-DMF and COX-2 under conditions similar to biological conditions over a period of time, molecular dynamic analysis was employed (Liu et al., 2018).

The molecular dynamic results denote a more stable structure of COX-2 bound to 5, 7-DMF compared to the bond with its inhibitor. The higher stability was indicated by lower Root Mean Square Fluctuation (RMSF) values and fewer fluctuations in the complex. RMSF is a measure of how much the position of atoms in the protein structure has changed during the simulation (Martínez, 2015; Ghahremanian et al., 2022). In this study, the RMSF of COX-2 bound with 5, 7-DMF was consistently below 3.5 Å, indicating that this complex is more stable compared to COX-2 bound with inhibitors having RMSF values exceeding 3.5 Å, where the fluctuation is also greater.

COX-2 is a gene involved in prostaglandin biosynthesis. Overexpression of COX-2 in cancer cells is closely associated with increased apoptosis resistance (Zarghi and Arfaei, 2011). COX-2 expression in cancer cells can promote tumor progression and create a microenvironment that suppresses the immune system, thereby inhibiting the immune response (Pu et al., 2021). Flavonoid compounds have been shown to have potential in inhibiting COX-2. Binding analysis shows that flavonoid compounds can interact with important amino acids in the COX catalytic domain which helps in inhibiting COX-2 activity (Idris et al., 2022).

COX-2 is frequently upregulated in breast cancer, influenced by ER-mediated modulation of Activated Protein-1 (AP-1) activity. Approximately 20-30% of breast tumors demonstrate HER-2 overexpression due to HER-2/neu gene amplification (Bartlett et al., 2003). In colorectal cancer cells, HER-2/neu activates COX-2 expression via the mitogen-activated protein kinase (MAPK) pathway (Hidalgo-Estévez et al., 2020), a mechanism similarly observed when HER-2/neu is transfected into breast cancer cells (Half et al., 2002). In ER-negative breast cells, COX-2 expression can be stimulated through Protein kinase C (PKC) and/or RAS/MAPK pathways (Bahar et al., 2023). High levels of COX-2 are linked to poor prognosis in breast cancer patients, correlating with larger tumor size and the presence of lymph node metastasis (Cui et al., 2017). The effectiveness of 5,7-DMF in inhibiting COX-2 highlights its strong potential as an anti-breast cancer agent.

This study revealed the potential of 5,7-dimethoxyflavone (5,7-DMF) as a promising anti-breast cancer agent by inhibiting kinase activity, acting as a competitive inhibitor of COX-2, and consequently suppressing breast cancer proliferation and development. Although 5,7-DMF is predicted to be non-toxic based on in silico toxicity screenings, this prediction does not fully capture the spectrum of potential adverse effects, particularly those relevant to long-term or clinical use. Currently, there is limited published data on its comprehensive toxicological profile, as most studies focus on its pharmacological activities rather than its safety. Given its structural similarity to other methoxylated flavones, several toxicity concerns warrant further investigation, including hepatotoxicity and nephrotoxicity, since flavonoids can sometimes affect liver and kidney function, especially with chronic exposure. Genotoxicity and mutagenicity should also be assessed using standard assays such as the Ames test and the micronucleus test to rule out the potential for DNA damage. Additionally, due to its methoxylation, 5,7-DMF may exhibit altered metabolism and a prolonged half-life, highlighting the need for comprehensive ADME (absorption, distribution, metabolism, and excretion) studies to evaluate any risk of bioaccumulation or drug interactions. To ensure its safety for clinical use, long-term toxicological assessments, including 28- or 90-day repeated dose toxicity tests, chronic toxicity evaluations, and specific dermal toxicity studies, are recommended following established guidelines.

Preclinical and in silico studies suggest that 5,7-DMF exerts anticancer effects by modulating key signaling pathways such as PI3K/Akt/mTOR, inducing apoptosis, causing cell cycle arrest, and inhibiting angiogenesis and metastasis (Li et al., 2017; Pandey et al., 2025). Beyond oncology, 5,7-DMF has also been reported to suppress sarcopenia by regulating protein turnover and mitochondrial biogenesis-related pathways (Kim and Hwang, 2020). Its antioxidant and anti-inflammatory activities hint at possible applications in inflammatory disorders such as rheumatoid arthritis and inflammatory bowel disease (Panthong et al., 1989). However, its clinical translation remains limited by poor water solubility and insufficient pharmacokinetic data, which could potentially be addressed through advanced drug delivery approaches such as nanoparticle formulations. While the compound demonstrates considerable promise, further in vivo studies and clinical trials are essential to fully establish its safety, efficacy, and therapeutic potential.

5. Conclusion

This study highlights the potential of 5,7-DMF, a compound found in *Piper ornatum*, as the promising anticancer agent against breast cancer, utilizing an in silico approach. The comprehensive analysis involved screening for drug-likeness, toxicity, membrane permeability, and bioactivity, alongside molecular docking and dynamic simulations. 5,7-DMF fulfils all criteria in drug-likeness screening, non-toxic to humans, penetrate to the plasma membrane easily, and act as a kinase activity inhibitor so it is predicted that 5,7-DMF has better anticancer activity. The results demonstrated that 5,7-DMF binds stably to the active site of COX-2 as a competitive inhibitor and also indicates potential as a kinase inhibitor, thereby underscoring its capability to inhibit breast cancer progression.

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