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In Silico Evaluation of *Typhonium flagelliforme* Fatty Acids for Cdc25B Inhibition and Chemotherapy Synergistic Potential

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Abstract: *Typhonium flagelliforme* exhibits cytotoxic activity against various cancer cell lines, including breast cancer. This study employed an in silico approach to evaluate the potential interaction or synergy between its fatty acid derivatives—2-octenoic acid and 2-hexenoic acid—and standard cytotoxic agents in breast cancer therapy. SwissTargetPrediction was used to identify the putative molecular targets of the compounds. Molecular docking was performed against phosphatase Cdc25B (PDB ID: 1CWR) using CB-Dock2, and pharmacokinetic properties were evaluated using SwissADME. Both 2-octenoic acid and 2-hexenoic acid were predicted to target Cdc25A and Cdc25B, which are key regulators of cell cycle progression. Molecular docking revealed binding affinities of −4.42 and −4.60 kcal/mol, respectively, compared to −5.97 kcal/mol for the native inhibitor NSC 663284. These compounds shared multiple key residues in the binding pocket, although they formed fewer hydrogen bonds. Pharmacokinetic predictions showed high gastrointestinal absorption, blood–brain barrier permeability, and no inhibition of major cytochrome P450 enzymes, suggesting minimal interaction with the metabolic pathways of standard chemotherapeutics. The results suggest that *T. flagelliforme* metabolites may not interfere with cytotoxic drug metabolism but could provide a synergistic effect by targeting cell cycle regulators.

Keywords: Cancer, Cdc25B, computational study, *Typhonium flagelliforme.*

Introduction

Cancer continues to be a leading cause of morbidity and mortality worldwide, reflecting not only the biological complexity of the disease, but also the global health burden it imposes. Among the various cancer types, breast cancer remains as one of the most prevalent malignancies. It is reported that over 2.3 million new cases diagnosed annually, making it the leading cause of cancer-related deaths among women (Sung et al., 2021). Despite advances in chemotherapeutic, hormonal, and targeted therapies, resistance to treatment and adverse side effects remain major challenges, particularly in patients receiving cytotoxic agents (Tufail, M. et al., 2022). Therefore, identifying adjunctive therapies that can improve efficacy or reduce toxicity is a priority in oncology research.

Medicinal plants have emerged as a promising source of bioactive compounds with anticancer properties. Their phytochemicals often act through multiple mechanisms, including cell cycle modulation, apoptosis induction, and inhibition of oncogenic pathways. Due to their relative safety profile, phytochemicals have attracted interest as complementary agents in to overcome resistance and toxicity in chemotherapy. In this context, "rodent tuber" plant or *Typhonium flagelliforme*, which traditionally used in Southeast Asian medicine, has drawn attention for its reported antiproliferative activity against various cancer cell lines, including breast, lung, and colon cancers (Swain, D. et al., 2021; Maha, HL. et al., 2023, Ng, KW. et al, 2023). However, the precise molecular mechanisms of its active constituents remain poorly defined.

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The application of computer-aided drug design (CADD) and in silico methods has accelerated the identification and characterization of bioactive molecules from natural products. These methods offers rapid and cost-effective strategy to explore the phytochemicals' therapeutic potential. While several studies have reported the anticancer activity of plant-derived compounds, many fail to elucidate their precise molecular targets or mechanisms of action in specific cancer models, particularly in breast cancer (Prabhu et al., 2024). This gap limits the translational value of such findings and impede with rational development of plant-based therapeutics. Molecular docking and target prediction tools can help address this by revealing how phytoconstituents interact with proteins involved in cancer progression (Ferreira et al., 2015; Aaron et al., 2025). Furthermore, ADME profiling supports early safety assessment by predicting drug-likeness and possible pharmacokinetic interactions, such as cytochrome P450 (CYP) enzyme inhibition, which is especially important when considering potential synergy with chemotherapeutic agents (Daina et al., 2017).

Given the pressing need for safer and more effective adjunctive therapies for breast cancer, this study aims to explore the potential of natural fatty acid derivatives as Cdc25B inhibitors. Specifically, we examined two unsaturated fatty acids 2-octenoic acid and 2-hexenoic acid previously identified in *Typhonium flagelliforme*, for their interaction with Cdc25B, a phosphatase implicated in cancer cell proliferation. Using an in silico approach, including target prediction, ADME profiling, and molecular docking, we assessed their pharmacological relevance and compared their binding behavior with NSC 663284, a known Cdc25B inhibitor, to evaluate their potential as supportive agents in breast cancer therapy.

Materials and Methods

Compound Selection, Target Prediction and Pharmacokinetic Profiling

Two secondary metabolites of *T. flagelliforme* extracts 2-octenoic acid and 2-hexenoic acid previously reported to exert cytotoxic activity were selected for computational study. To estimate biological targets of the metabolites, SwissTargetPrediction (http://www.swisstargetprediction.ch/) was used (Daina et al., 2019). SMILES strings of 2-octenoic acid and 2-hexenoic acid were input to predict potential human protein targets. SwissTargetPrediction estimated phosphatases like Cdc25A/B to be the most probable target of the compounds, hence was selected to be the target protein for molecular docking study. Pharmacokinetic and drug-likeness properties were predicted using SwissADME (http://www.swissadme.ch/), focusing on GI absorption, BBB permeability, CYP450 inhibition, P-glycoprotein interaction, and skin permeability (Log Kp) (Daina et al., 2017).

Compound Preparation

The chemical structures of 2-octenoic acid and 2-hexenoic acid were retrieved from the PubChem database or drawn manually using PubChem Sketcher. Files were saved in .mol format and converted to .pdb format using CADD Chemical Identifier Resolver tools. The reference compound, NSC 663284, a known inhibitor of the dual-specificity phosphatase Cdc25B, was included for comparison.

Target Protein Preparation

The crystal structure of Cdc25B phosphatase (PDB ID: 1CWR) in complex with NSC 663284 was obtained from the RCSB Protein Data Bank. The protein was prepared by removing all non-receptor molecules (co-crystallized ligand, solvent molecules, and ions) using UCSF Chimera, and hydrogen atoms were added to stabilize the structure.

Molecular Docking

Docking simulations were conducted using CB-Dock2 (http://cadd.labshare.cn/cb-dock2/php/), a web-based blind docking tool that automatically identifies binding cavities and runs docking via AutoDock Vina (Yang et al., 2022; Liu et al., 2022). Each compound was uploaded in .pdb format, and CB-Dock2 provided up to five docking poses per ligand. The pose with the lowest binding energy and binding site overlap with the native ligand was selected for further analysis.

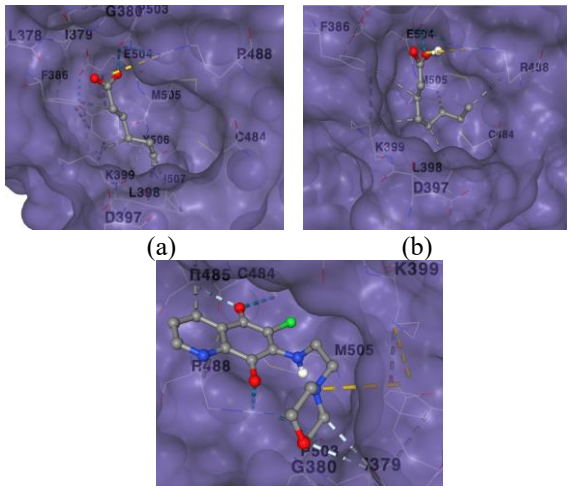
Binding Site Comparison and Interaction Analysis

Protein–ligand binding residues were extracted directly from the docking output. Residue interactions for each compound were manually compared to those of NSC 663284, focusing on amino acids within the ligand-binding pocket of Cdc25B as identified from the co-crystal structure. The number and nature of predicted hydrogen bonds and overlapping residues were used to qualitatively assess binding similarity.

Results and Discussion

Binding Affinity of the Compounds Against Cdc25B

SwissTargetPrediction identified Cdc25A and Cdc25B as the top predicted targets for both 2-hexenoic acid and 2-octenoic acid, suggesting that these phosphatases may serve as the primary sites of action for the compounds. In this study, molecular docking was used to predict how well two compounds from *Typhonium flagelliforme* — 2-octenoic acid and 2-hexenoic acid — could bind to Cdc25B, a cell cycle-regulating phosphatase associated with tumorigenesis and cancer progression. Their performance was compared with NSC 663284, a known inhibitor of Cdc25B. The docking results showed that NSC 663284 had the strongest predicted binding, with a binding affinity of −5.97 kcal/mol. On the other hand, compared to the previous compound, 2-hexenoic acid had moderate binding affinity of −4.60 kcal/mol and 2-octenoic acid had the weakest binding, −4.42 kcal/mol. The comprehensive docking results are presented in table 1 and figure 1.



(a) (b)

(c)

Figure 1. Compound interaction with Cdc25B, (a) 2-hexenoic acid, (b) 2-octenoic acid, and (c) NSC 663284

Table 1. Predicted Binding Affinity of *Typhonium flagelliforme*-Derived Fatty Acids Against Cdc25B

Compound	Binding Affinity (kcal/mol)	No. of Hydrogen Bonds	Key Interacting Residues
NSC 663284	−6,7	2	GLU377, LEU378, ILE379, GLY380, TYR382, PHE386, ASP397, LEU398, LYS399, CYS484, ARG485, ARG488, GLU489, ARG492, TYR497, PRO503, GLU504, MET505, TYR506
2-Octenoic Acid	−5.0	1	GLU377, LEU378, ILE379, GLY380, TYR382, PHE386, ASP397, LEU398, LYS399, CYS484, ARG485, ARG488, GLU489, ARG492, LEU500, TYR502, PRO503, GLU504, MET505, TYR506, ILE507
2-Hexenoic Acid	−4.60	1	GLU377, LEU378, ILE379, GLY380, ASP381, PHE386, ASP397, LEU398, LYS399, PRO481, CYS484, ARG485, PHE486, ARG488, LEU500, TYR502, PRO503, GLU504, MET505, TYR506, ILE507

Pharmacokinetic Properties of Typhonium flagelliforme-Derived Fatty Acids

The pharmacokinetic properties of 2-octenoic acid and 2-hexenoic acid were predicted using SwissADME. The results for both compounds are summarized in the table 2 below: Both compounds show high oral absorption and the ability to cross the blood-brain barrier, indicating good bioavailability and systemic distribution potential. Importantly, neither compound is

predicted to inhibit major CYP450 enzymes or act as P-glycoprotein substrates, suggesting a low risk of drug-drug interactions when co-administered with standard cytotoxic agents. These pharmacokinetic characteristics support their potential as safe adjunctive agents in combination cancer therapy.

Table 2. Predicted Pharmacokinetic Properties of *Typhonium flagelliforme*-Derived Fatty Acids

Property	2-Octenoic Acid	2-Hexenoic
GI Absorption	High	High
BBB Permeability	Yes	Yes
P-gp Substrate	No	No
CYP1A2 Inhibition	No	No
CYP2C9 Inhibition	No	No
CYP2C19 Inhibition	No	No
CYP2D6 Inhibition	No	No
CYP3A4 Inhibition	No	No
Skin Permeation	−5.27 cm/s	−5.87 cm/s
Bioavailability Score	0.85	0.85

Discussion

This study evaluated whether two secondary metabolites derived from *Typhonium flagelliforme*, 2-octenoic acid and 2-hexenoic acid, could interfere with or synergize with the action of conventional cytotoxic agents used in breast cancer therapy. These compounds were previously reported to exhibit cytotoxic activity toward breast cancer cell line MCF7 (Sianipar et al., 2023). This study extends prior research through the application of molecular docking, pharmacokinetic predictions, and target profiling to assess both interaction potential and drug-likeness.

Target Prediction and Molecular Specificity

SwissTargetPrediction analysis revealed that both 2-octenoic acid and 2-hexenoic acid are likely to interact with dual-specificity phosphatases Cdc25A and Cdc25B. These enzymes play essential roles in regulating cell cycle progression by activating cyclin-dependent kinases (CDKs). Aberrant overexpression of Cdc25 family members has been associated with uncontrolled proliferation and therapy resistance in breast cancer (Sur et al., 2016; Al-Matouq et al., 2019). Interestingly, neither of the compounds demonstrated predicted activity against tubulin, topoisomerase II, or other typical targets of standard cytotoxic agents such as doxorubicin or paclitaxel, suggesting a distinct mechanism of action. This opens the possibility of non-competitive synergy when co-administered with standard therapies.

Molecular Docking Analysis

Explore direct binding interaction with the Cdc25B protein, molecular docking was conducted using CB-Dock2, targeting the crystal structure of human Cdc25B phosphatase (PDB ID: 1CWR). The known inhibitor NSC 663284 was used as a reference ligand for benchmarking. The docking results showed that the predicted binding affinity of 2-octenoic acid, 2-hexenoic acid, and the reference ligand, NSC 663284, were -4.42 kcal/mol, -4.60 kcal/mol, and -5.97 kcal/mol, respectively. While both natural compounds showed weaker binding than the reference, their docking poses revealed notable overlap in key Both compounds show high oral absorption and the ability to cross the blood-brain barrier, indicating good bioavailability and systemic distribution potential.

Importantly, neither compound is predicted to inhibit major CYP450 enzymes or act as P-glycoprotein substrates, suggesting a low risk of drug-drug interactions when co-administered with standard cytotoxic agents. These pharmacokinetic characteristics support their potential as safe adjunctive agents in combination cancer therapy. Interacting residues. Specifically, residues such as GLU377, LEU378, ILE379, GLY380, ASP397, LEU398, LYS399, CYS484, ARG485, ARG488, GLU504, MET505, TYR506, and ILE507 were commonly involved in binding for both test compounds and NSC 663284.

Hydrogen bond formation, an important factor in ligand stability and specificity, was observed to be limited to a single hydrogen bond in both 2-octenoic and 2-hexenoic acid, compared to two in the native ligand. However, the presence of hydrophobic and van der Waals contacts, particularly through the aliphatic chains of the fatty acids, may partially compensate for the lack of polar interactions. Notably, 2-hexenoic acid engaged additional residues such as PRO481, PHE486, and TYR502, indicating a

slightly altered binding orientation that may reflect functional differences in inhibitory activity.

Pharmacokinetic Properties and Drug Interaction Risk

ADME (Absorption, Distribution, Metabolism, and Excretion) analysis using SwissADME showed that 2-octenoic acid and 2-hexenoic acid possessed favorable pharmacokinetic features, as shown on table 2. It demonstrated high gastrointestinal absorption, was blood-brain barrier (BBB) permeable, and was not a substrate for P-glycoprotein. Importantly, it did not inhibit major cytochrome P450 enzymes (CYP1A2, 2C19, 2C9, 2D6, 3A4), indicating low risk of metabolic drug-drug interactions when co-administered with conventional anticancer drugs. These properties support the potential oral bioavailability of the compound and reduce the likelihood of adverse interactions with chemotherapeutic agents metabolized via the CYP pathway.

Potential for Synergistic Action

Given their distinct molecular targets, minimal CYP inhibition, and overlap with active site residues of a known Cdc25 inhibitor, both test compounds-especially 2-hexenoic acid-could function as adjunctive agents. Prior study have reported that overexpression of Cdc25B in hepatocellular carcinoma promotes uncontrolled proliferation and drug resistance through activation of cell cycle and DNA repair pathways, while simultaneously facilitating immune evasion via modulation of the tumor microenvironment and immune checkpoint upregulation (Huang et al., 2024). By inhibiting Cdc25B, these metabolites may contribute to cell cycle arrest and potentially enhance sensitivity to chemotherapeutics and improve the patient's prognosis (Kabakci et al., 2019; Liu, K. et al., 2020; Wang et al., 2023). Although in silico predictions do not confirm biological activity, the observed binding behavior and target specificity provide a rational basis for further in vitro or in vivo validation.

Limitations

This study is based entirely on computational models. Docking scores provide only approximate estimations of binding affinity and do not account for real-time molecular dynamics or cellular bioavailability. Moreover, hydrogen bonding predictions, while informative, do not confirm functional inhibition without biochemical assays. Therefore, further experimental studies, such as enzyme inhibition assays, cancer cell viability tests, or drug combination experiments, are essential to validate these findings.

Conclusion

This study explored the potential role of *Typhonium flagelliforme* secondary metabolites specifically 2-octenoic acid and 2-hexenoic acid as modulators of cell cycle progression through in silico analysis. Although these compounds do not directly target the common molecular pathways of widely used cytotoxic agents in breast cancer, their predicted affinity toward Cdc25 phosphatases suggests a complementary mechanism of action. The combination of favorable pharmacokinetic profiles, minimal interaction with major drug-metabolizing enzymes, and shared binding residues with known Cdc25B inhibitors highlights their potential as non-interfering, possibly synergistic adjuncts in cancer therapy.

While the computational findings are promising, they serve primarily as a foundation for future investigation. Experimental validation through enzymatic assays, cellular studies, and synergy testing with standard chemotherapeutics will be essential to confirm these initial predictions and assess therapeutic relevance.

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