INVESTIGATION OF A POTENTIAL NATURAL INHIBITOR AGAINST HUMAN OPIOID BINDING PROTEIN/CELL ADHESION MOLECULE LIKE (OPCML) IN OVARIAN CANCER

Rory Anthony Hutagalung[a,*], Giovani[a], Rosmalena Rosmalena[b], Kristina Simanjuntak[c], Ernawati Sinaga[d], Vivitri Dewi Prasasty[d,*]

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Abstract

Background:

Ovarian cancer, a formidable threat to women's health, is characterized by its high prevalence and the challenges associated with its diagnosis and treatment. As one of the leading causes of gynecological cancer-related mortality, ovarian cancer necessitates novel therapeutic approaches that address its aggressive nature and limited treatment options. Our study aimed to elucidate the binding affinity and conformational aspects of OPCML with natural inhibitors, offering a comprehensive understanding of their potential therapeutic impact. **Methods:** Molecular docking simulations were performed to assess the binding energies and interactions within the active site of the OPCML receptor complex. Ligands, such as orientin, were selected based on their diverse chemical properties and structural characteristics. Computational analyses involved a comprehensive approach, considering the potential therapeutic impact of each ligand. **Results:** Orientin performed as a standout candidate, displaying the best affinity binding with the lowest energy within the OPCML receptor complex's active site. The favorable binding energy indicates a robust and stable interaction with the receptor, showcasing orientin's potential as an effective OPCML ligand. However, the computational nature of molecular docking necessitates cautious interpretation, emphasizing the need for further experimental validations. **Conclusion:** While orientin demonstrates promising affinity binding, further in vitro and in vivo studies are essential to confirm its actual binding activity with OPCML and assess its translational potential as a therapeutic agent. The diverse set of ligands provides a comprehensive overview of potential OPCML ligands, underscoring the importance of a broad screening approach in drug discovery. The identification of orientin as the lead candidate emphasizes the significance of such an approach. This molecular docking study opens avenues for further research into orientin's mechanisms of action and therapeutic efficacy, laying the groundwork for targeted experimental validations that advance our understanding of orientin as a potential OPCML ligand with biological and therapeutic relevance.

Keywords: Opioid binding protein/cell adhesion molecule like (OPCML) ; molecular docking analyses ; ovariab cancer ; computational drug discovery ; orientin

[a]. Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jakarta 12930, Indonesia;
[b]. Department of Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta 10440, Indonesia;
[c]. Department of Biochemistry, Faculty of Medicine, UPN Veteran Jakarta, Jakarta 12450, Indonesia;
[d]. Faculty of Biology and Agriculture, Universitas Nasional, Jakarta 12520, Indonesia.

***Corresponding author:** Rory Anthony Hutagalung (email: rory.hutagalung@atmajaya.ac.id) ; Vivitri Dewi Prasasty (email: vivitri.prasasty@unas.ac.id)

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INTRODUCTION

Ovarian cancer (OC), while not as common as some other cancers, still affects a significant number of women worldwide. Around the globe, an estimated 239,000 new cases are diagnosed annually, making it the seventh most common cancer among women [1]. The average annual ovarian cancer incidence rate in the United States was 11.5 per 100,000 women during 2010 through 2014 [2]. The detection of this cancer has been problematic as there is no screening public program for its early detection and as a consequence, most OCs are detected in an advanced stage where most of the time it has already spread to other parts different from the ovary [3].

Human opioid binding protein/cell adhesion molecule like (OPCML) is a protein that plays a crucial role in various cellular processes, including cell adhesion, migration, and invasion [4]. Studies have shown that OPCML expression is downregulated in ovarian cancer cells, suggesting its potential role as a tumor suppressor [5]. This has led to increasing interest in targeting OPCML for the treatment of ovarian cancer. Several therapeutic strategies are being explored to target OPCML for ovarian cancer treatment such as gene therapy [6]. This approach involves delivering the OPCML gene directly into cancer cells to increase its expression.

Preclinical studies have shown promising results, with increased OPCML expression leading to tumor regression and improved survival in animal models. Other treatments are small molecule drugs that can mimic the functions of OPCML and suppress tumor growth [7]. These drugs work by binding to specific domains of the OPCML protein and activating its tumor suppressor activity. Antibody-based therapy using monoclonal antibodies designed to target OPCML are being developed to specifically attack cancer cells that express low levels of OPCML [8]. These antibodies can also deliver therapeutic agents, such as chemotherapy drugs or toxins, directly to cancer cells. However, many challenges will be faced in regards of the safety and efficacy of OPCML-targeting therapies in humans.

Embarking on a journey into the realm of ovarian cancer therapeutics, this study delves into the promising potential of employing natural inhibitors against human opioid binding protein/cell adhesion molecule like (OPCML). Central to our investigation, our study aimed to seamlessly encapsulate the pivotal focus of our research. Employing advanced molecular docking techniques, by unraveling the interaction between OPCML and potential natural inhibitors, enlightening pathways for the development of innovative and precisely targeted interventions against ovarian cancer. In this research endeavor, our goal is to make a compelling contribution to the ever-evolving landscape of ovarian cancer treatment, offering a nuanced understanding of OPCML inhibition as a promising and sophisticated therapeutic avenue.

MATERIALS AND METHODS

Materials

The receptor complex of human Opioid binding protein/cell adhesion molecule like (OPCML) was obtained from the Protein Data Bank Repository (PDB) with the identifier: 5UV6 [9]. The corresponding files, in .pdb format, were downloaded. Additionally, a 3D file conformer of the commercial drug Gemcitabine, original ligand of NAG and 20 ligand files including 7-methoxycoumarin, cedrusin, isoorientin, dulcisflavan, myrianthic acid, gallic acid, euscaphic acid, orientin, tectoridin, ginnalin A, ursolic acid, epicatechin, epigallocatechin, 3,4-dihydroxybenzoic acid, tormentic acid, pinoresinol, madecassic acid, arjunolic acid, vanillic acid were downloaded from PubChem [10]. These ligand files were in .sdf format.

Protein Preparation and Virtual Screening

The .pdb files of the protein, after removing the initial ligands and water molecules with Discovery Studio Visualizer. Preparing a protein structure for molecular docking with PyRx involves several crucial steps to ensure the protein's suitability for docking simulations. These steps include obtaining the protein structure in a compatible format, loading it into PyRx, removing water molecules, adding missing residues if necessary, adding hydrogen atoms, assigning atom types (optional), optimizing the structure (optional), and saving the prepared protein structure. By following these steps, researchers can ensure that their protein structures are adequately prepared for accurate and reliable docking simulations.

Molecular Docking

The protein and ligand were prepared using PyRx Tools software and converted into .pdbqt format [11]. To perform molecular docking simulations, follow these steps: Begin by obtaining the protein structure from a database like the Protein Data Bank (PDB) and load it into PyRx. Prepare the protein by removing water molecules, adding missing residues, and incorporating hydrogen atoms. Optionally, assign atom types and optimize the structure for enhanced accuracy. Save the prepared protein structure in PDB or PDBQT format. Next, obtain the ligand structure from a chemical database or create it computationally, ensuring compatibility with PDB or SDF formats. Load the ligand into PyRx, add hydrogen atoms if needed, assign atom types, and convert to PDBQT format if necessary. Save the prepared ligand structure in a suitable format. These processed structures are then ready for subsequent docking simulations. The utilization of the binding energy (ΔG) value allowed for the quantification of the strength of interaction between the ligand and the target in the molecular docking process. Moreover, determining inhibition constants (Ki) involved evaluating the binding strength between a ligand and a target receptor. This assessment was carried out by applying the formula:

$$Ki = e^{-RT/\Delta G}$$

Protein and Ligand Interaction

The formation of protein and ligand docking data was done to adhere to .pdb files. The PyRx program was employed for the data integration process to ensure a standardized and coherent representation for subsequent analyses. Moreover, PyMOL was utilized for a structured 3D visualization, enabling a meticulous exploration of spatial arrangements, binding interfaces, and conformational changes [12].

RESULTS

Protein and Ligand Interaction

This analysis employed Pyrx's gridbox tool to define the receptor docking region for molecular docking. Figure 2 depicted the binding energy and 3D interactions between the human Opioid binding protein/cell adhesion molecule like (OPCML) and the investigated ligand inhibitors, orientin emerged as the compound with the most favorable binding affinity among the 18 tested, demonstrating promising potential for further investigation. Notably, the docking process was validated using the original ligand (NAG) obtained from the protein-ligand complex 3D structure, confirming the accuracy of the simulations.

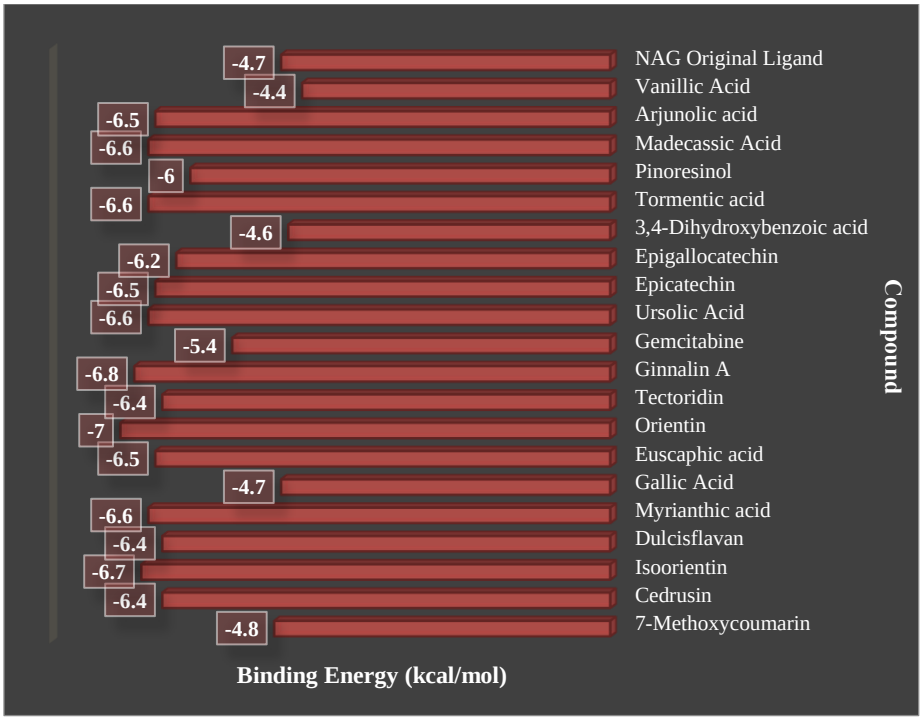


Figure 1: Binding energy between natural compounds and Opioid binding protein/cell adhesion molecule like (OPCML).

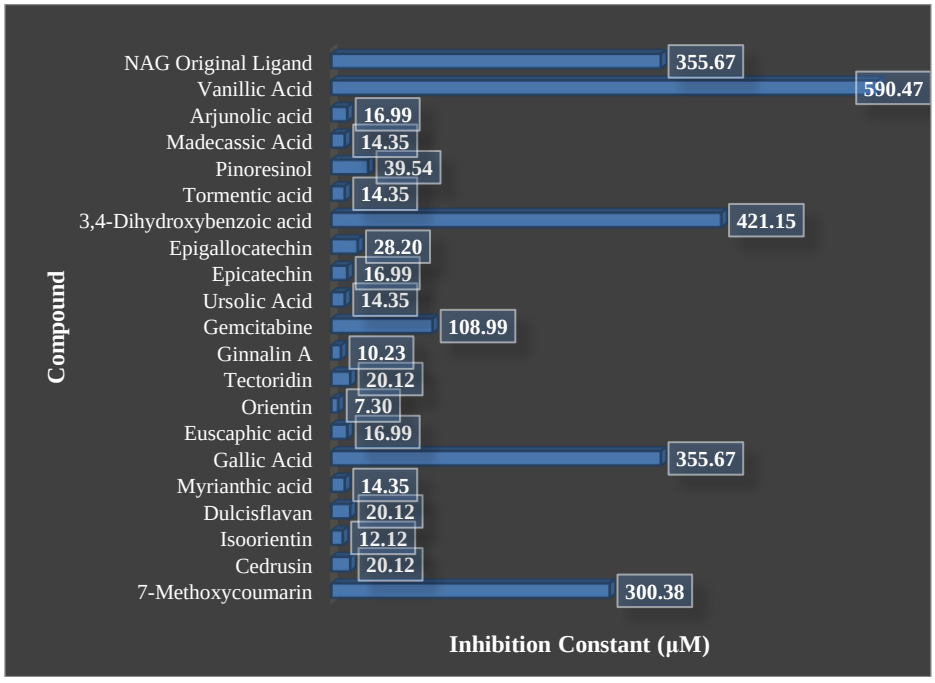
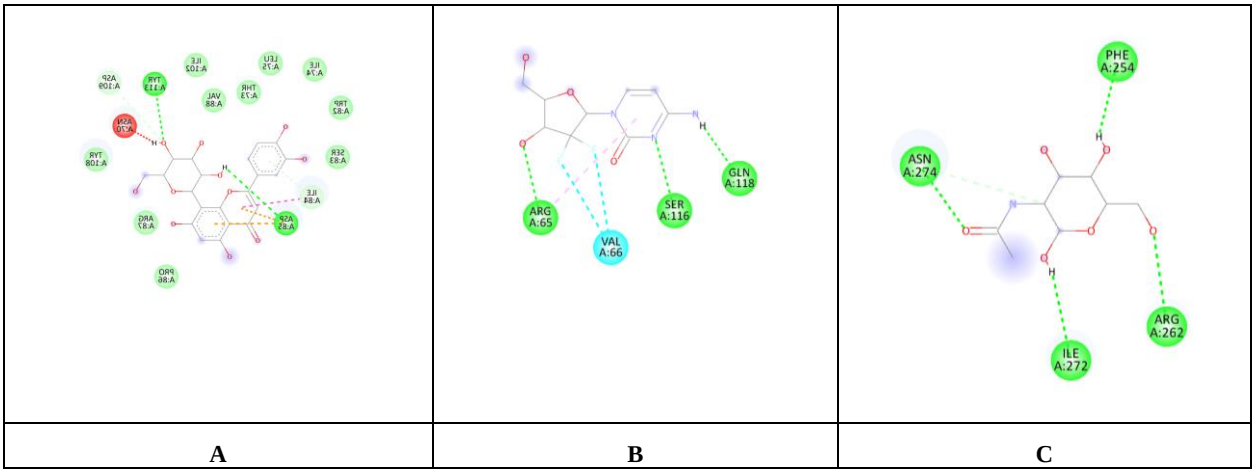


Figure 2: Inhibition constant of natural compounds towards opioid binding protein/cell adhesion molecule like (OPCML).

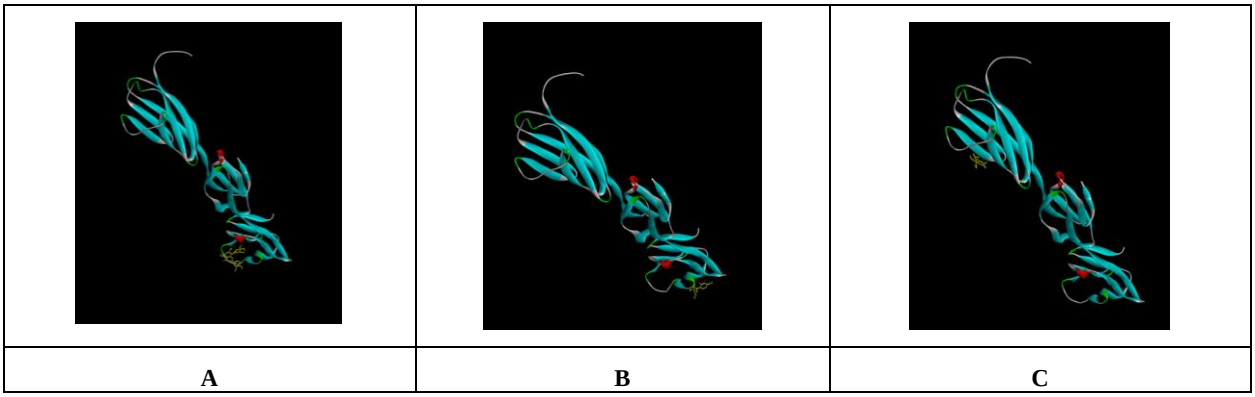
Figures 3 and 4 present detailed 2D and 3D visualizations illustrating interaction complexes involving opioid binding protein/cell adhesion molecule like (OPCML) with two distinct entities: orientin and the pharmaceutical drug gemcitabine. The figures also depict the interaction between OPCML and its original ligand, offering a comprehensive comparison of their binding characteristics. In panel A, the intricate interaction between OPCML and orientin is visually represented, providing molecular insights into their binding details. This visualization aids researchers in discerning specific sites and bonding patterns involved in the interaction,

shedding light on potential pharmacological implications of orientin with OPCML. Moving to panel B, the figure portrays the interaction between OPCML and the chemotherapeutic drug gemcitabine, facilitating a comparative analysis of binding features between gemcitabine and OPCML in contrast to the interaction with orientin. Understanding these interactions contributes to assessing the potential therapeutic impact of gemcitabine on OPCML. Finally, in panel C, the figure includes the interaction between OPCML and its original ligand, serving as a baseline for comparison. This reference point facilitates a thorough examination of any deviations or enhancements in the binding patterns introduced by orientin or gemcitabine



A **B** **C**

Figure 3: The 2D visualization of interactions complex between: A. Opioid binding protein/cell adhesion molecule like (OPCML) and the Orientin, B. OPCML and gemcitabine drug, OPCML and the original ligand.



A **B** **C**

Figure 4: The 3D visualization of interactions complex between: A. Opioid binding protein/cell adhesion molecule like (OPCML) and the orientin, B. OPCML and gemcitabine drug, OPCML and the original ligand.

DISCUSSION

The molecular docking results provide crucial insights into the potential interactions between the human Opioid Binding Protein/Cell Adhesion Molecule Like (OPCML) receptor complex and a diverse set of compounds. The OPCML receptor complex, sourced from the Protein Data Bank (PDB) with the identifier 5UV6, served as the structural foundation for these investigations [11]. The molecular docking analysis involved a 3D conformer of the commercial drug Gemcitabine, the NAG Original ligand, and 20 ligand files encompassing various natural compounds.

Among the tested compounds, orientin emerged as a notable candidate, demonstrating the best affinity binding with the lowest binding energy within the active site of the OPCML receptor complex. The favorable binding energy of orientin suggests a robust and stable interaction with the receptor, indicating its potential as an effective ligand for OPCML. This finding is significant as it highlights orientin's capacity to form strong molecular interactions within the binding pocket of the receptor.

However, it is crucial to interpret these results judiciously, considering the computational nature of molecular docking. While orientin showed the best affinity binding, further experimental validations are imperative to confirm its actual

binding activity with OPCML. In vitro and in vivo studies will be essential to corroborate the computational findings and assess the translational potential of orientin as a potential therapeutic agent.

The diverse set of ligands, including 7-methoxycoumarin, cedrusin, isoorientin, dulcisflavan, myrianthic acid, gallic acid, euscaphic acid, tectoridin, ginnalin A, ursolic acid, epicatechin, epigallocatechin, 3,4-dihydroxybenzoic acid, tormentic acid, pinoresinol, madecassic acid, arjunolic acid, and vanillic acid, provides a comprehensive overview of potential ligands for OPCML. The identification of orientin as the lead candidate emphasizes the importance of employing a broad screening approach to discover novel ligands with therapeutic potential.

Orientin is a flavonoid compound found in various plants, particularly in the Orientin- rich extract of the plant, *Passiflora incarnata* [13]. Numerous studies have explored the biomedical activities of orientin, revealing a range of potential health benefits. Orientin possesses significant antioxidant activity, helping to neutralize harmful free radicals in the body [14]. This property is crucial for protecting cells from oxidative stress, which is implicated in various chronic diseases. Some studies have explored the potential anticancer effects of orientin, demonstrating its ability to inhibit the growth of certain cancer cells [15, 16]. However, more research is needed to fully understand its mechanisms and clinical applications.

The molecular docking method sheds light on the promising affinity binding of orientin to the OPCML receptor complex. This result opens avenues for further research, encouraging detailed investigations into orientin's mechanisms of action and therapeutic efficacy. The findings from this molecular docking study lay the groundwork for targeted experimental validations, advancing our understanding of orientin as a potential ligand against OPCML in the context of its biological and therapeutic relevance.

CONCLUSION

In conclusion, orientin emerged as a noteworthy candidate, displaying the most favorable affinity binding with the lowest binding energy within the OPCML receptor complex's active site. The robust interaction and promising binding energy of orientin suggest its potential as an effective ligand for OPCML, showcasing its capability to form stable molecular interactions within the receptor's binding pocket. This result encourages detailed investigations into orientin's mechanisms of action and therapeutic efficacy. The findings from this molecular docking study serve as a foundation for targeted experimental validations, advancing our understanding of orientin as a potential ligand against OPCML in the biological and therapeutic context.

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