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Virtual Screening and Comparison of the Binding Effectiveness of Doxorubicin, Paclitaxel, and Ergoloid to ERβ-MDM2 Complex Protein as New Breast Cancer Drug Candidates

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ABSTRACT

Background: The prevalence of breast cancer continues to increase, it is the second leading cause of death after lung cancer. Estrogen receptor beta (ERβ) has an important role in breast cancer pathology, activation of Akt pathway will trigger E3 ubiquitin ligase murine double minute 2 (MDM2) to ERβ, which will increase the risk of breast cancer. Thus, both proteins have potential as therapeutic agents in breast cancer drug development. This study aims to find breast cancer drug candidates from natural products and compare the effectiveness of these compounds with Doxorubicin and Paclitaxel.

Method: ERβ (PDB ID: 3OLS) and MDM2 (PDB ID: 1T4E) proteins were combined using ClusPro 2.0. Doxorubicin and Paclitaxel ligands were obtained from PubChem, there are 842 natural products obtained from the ZINC database, when the energy minimization is reduced to 839 natural products. Virtual screening between proteins and ligands was performed using PyRx 8.0, followed by analysis of amino acid residues resulting from interactions between proteins and ligands using protein interaction calculator (PIC) and protein ligand interaction profiler (PLIP).

Results: Ergoloid compounds have the lowest binding affinity compared to doxorubicin and paclitaxel compounds, and are able to interact strongly with the ERβ-MDM2 Protein as determined from the results of interactions between proteins and ligands using PIC and PLIP.

Conclusion: Ergoloid compounds can interact well with ERβ-MDM2 Protein. Thus, it can be used as a breast cancer drug candidate in the future. In vitro, in vivo, and biochemical testing needs to be done to confirm this discovery.

INTRODUCTION

Cancer is a degenerative disease that continues to experience an increase in cases every year and is positioned as the second cause of death [1]. Breast cancer is the second highest prevalence of cancer after lung cancer [2]. According to data [3], there were 19.3 million global cancer cases, of which 2.2 million or about 11.7% were breast cancer cases [4]. In Indonesia, cancer is also a significant source of death. WHO data for 2020 shows that in 2018 there were 207, 210 cancer deaths, of which breast cancer had the largest proportion, covering 11% of the total cases [2].

As many as 70% of breast cancer cases show high expression of estrogen receptor (ER) [5–6]. Estrogen is a major signal that plays a major role in tumor cell growth and development. The cellular mechanism of estrogen is mediated by nuclear ERα, ERβ and G membrane protein-coupled ER (GPER, also called GPR30) [5]. These receptors also play a role in assessing the sensitivity of

anti-estrogen therapy to breast cancer as well as for evaluating the effectiveness of preventive chemotherapy in individuals at high risk of developing breast cancer [7]. ERβ is closely associated with increased cell proliferation and disease prognosis [8]. ER-β isoforms are found in 20–30% of breast cancer cases [9–10]. Activation of the phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (AKT) pathway acts as an important mechanism of ERβ downregulation in breast cancer and is thought to be associated with phosphatase and tensin homolog deleted on chromosome ten (PTEN) [11–12]. Increased PI3-K/Akt signaling and synergistic activation of cyclic adenosine monophosphate (cAMP) response element-binding (CREB)-binding protein (CBP) induce ERβ ubiquitination and degradation. This process is amplified by the negatively charged hinge region of ERβ. Activated Akt triggers the recruitment of E3 ubiquitin ligase MDM2 to ERβ, which is further stabilized by CBP, resulting in ERβ polyubiquitination [13]. By increasing PTEN levels and decreasing human epidermal growth factor receptor 2 (HER2) or human epidermal growth factor receptor 3 (HER3) protooncogene signaling, Akt signaling is reduced [14].

This opens up opportunities for breast cancer drug development by making the interaction between ERβ and MDM2 a therapeutic target. The concept is to create molecules that can stabilize the interaction between ERβ and MDM2, triggering more 'free' MDM2 to bind to ERβ. This would reduce the expression of MDM2 and its interaction with p53, with the hope of halting cancer cell growth [10]. Natural products have the potential to reduce breast cancer mortality and improve outcomes compared to conventional breast cancer treatment [15]. Anticancer effects are significantly shown by natural products by modulating cellular processes such as angiogenesis, proliferation, differentiation, invasion, migration, and metastasis [16]. This study conducted a virtual screening of Natural Products obtained from the ZINC Database to find compounds that can stabilize the ERβ-MDM2 bond. ClusPro 2.0 was used for docking ERβ and MDM2 proteins, thus obtaining the ERβ-MDM2 complex protein. Protein Interaction Calculator (PIC) and Protein Ligand Interaction Profiler (PLIP) were used to analyze the interaction between Doxorubicin, Paclitaxel, natural product as ligand and ERB-MDM2 as protein. This study aims to find breast cancer drug candidates from natural products and compare the effectiveness of these compounds with Doxorubicin and Paclitaxel.

METHODS

The research design used is experimental research with the in silico method. The hardware used is Asus Intel (R) Core (TM) i5-5200U CPU @ 2.20GHz (4 CPUs) 5 GB RAM. Software used are ClusPro, PIC, PLIP, PyMOL, and BIOVIA Discovery Studio.

Protein and ligand preparation

The 3D structures of ERβ (PDB ID: 3OLS) and MDM2 (PDB ID: 1T4E) proteins were downloaded from Protein Data Bank (https://www.rcsb.org/). Protein preparation was performed to remove water molecules, natural ligands, and other molecules using Biovia Discovery Studio 2021. ClusPro 2.0 was used for docking the prepared proteins. Doxorubicin and Paclitaxel were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and natural products (Ergoloid) are obtained from the ZINC Database, natural products are also known as secondary metabolites. There are 842 natural products obtained from the ZINC database, when the energy minimization is reduced to 839 natural products. The ligands were prepared using PyRx 8.0 by minimizing their energy.

Docking of ERβ and MDM2 proteins

ClusPro 2.0 is an online application used for protein-protein docking. Therefore, it can be used for docking ERβ and MDM2. Cluster data, members, and weighted score will be used as a comparison in the selection of ERβ-MDM2 protein complex [17]. MDM2 protein has a dimer structure consisting of A and B chains. So, the results of protein-protein docking between ERβ-MDM2 chain A and ERβ-MDM2 chain B will be compared. PIC web server (http://pic.mbu.iisc.ernet.in/) which is used to find out the amino acid residues contained in ERβ-MDM2, and FTMap (https://ftmap.bu.edu/login.php) is used to predict "hot spots" in the ERβ-MDM2 protein complex.

Virtual screening of ligands with ERβ and MDM2 complex proteins

Virtual screening is only carried out on Natural Product compounds from the ZINC Database which will be docked to the ERβ-MDM2 complex protein using PyRx 8.0. There are 842 compounds that can be read by Open Babel, but after energy minimization, there are 839 compounds that can be virtually screened. The grid box used in the virtual screening process is Center X: 18.4925 Y: -23.092 Z:4.7850 Dimensions (Angstrom) X: 74.1088 Y:60.8562 Z: 73.9596. The results of virtual screening in the form of binding affinity and root mean square deviation (RMSD), in this study will be taken one compound that has the lowest binding affinity value, then will be visualized using PyMOL.

RESULTS

ERβ and MDM2 protein-protein interaction

The results of protein-protein docking using ClusPro 2.0 show that the best bond between ERβ and MDM2 (chain A and B) is ERβ-MDM2 chain B **(Table 1)**. The ERβ-MDM2 complex protein model will be continued for the virtual screening stage using PyRx 8.0 **(Figure 1)**. Analysis using PIC yielded the following results. From the PIC results, the amino acid residues varied **(Table 2).**

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Table 1. Comparison of the lowest energy of estrogen receptor β-murine double minute2 chain A (ERβ-MDM2 A) and estrogen receptor β-murine double minute2 chain B (ERβ-MDM2 B)

Cluster	Member	Representative	Weighted score	Protein complex
0	63	Center	-614.5	ERβ-MDM2 A
		Lowest energy	-649.3	
*1	81	Center	-627.8	ERβ-MDM2 B
		Lowest energy	-761.0	

* ERβ-MDM2 B complex protein selected for virtual screening using PyRx 0.8 software
ERβ-MDM2 A: Estrogen receptor β-murine double minute2 chain A,
ERβ-MDM2 B: estrogen receptor β-murine double minute 2 chain B.

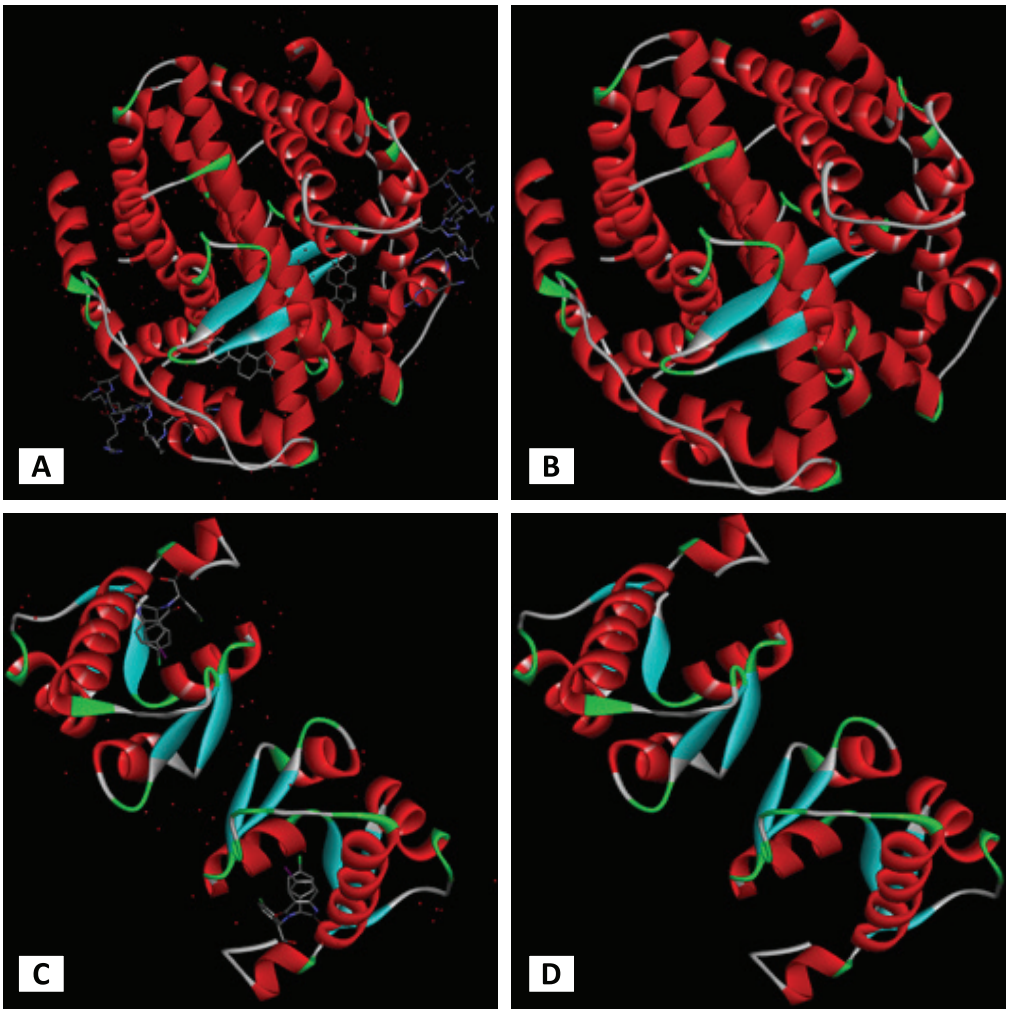

Figure 1. Visualization of 3OLS protein before (A) and after (B) preparation; 1T4E protein before (C) and after (D) preparation.

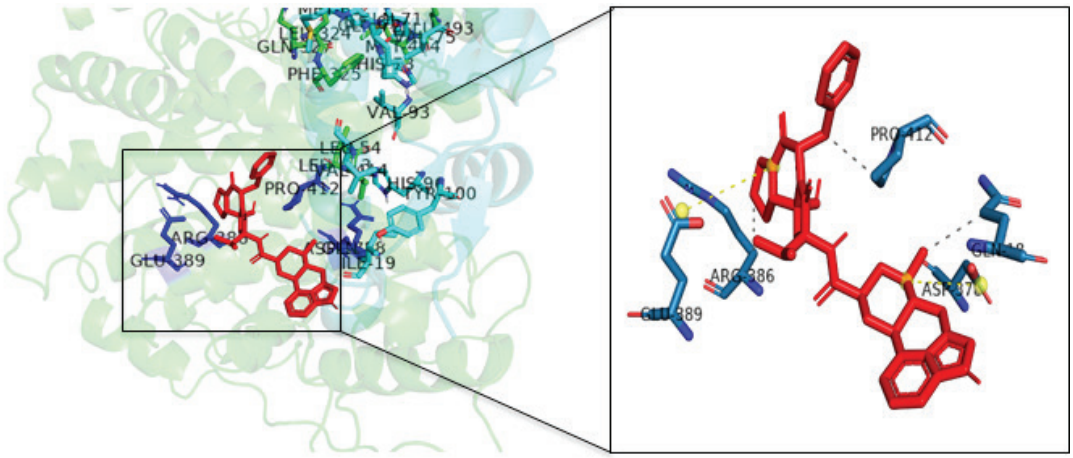

Figure 2. Amino acids resulting from the interaction between estrogen receptor β-murine double minute 2 chain B (ERβ-MDM2 B) (cyan and green), amino acids resulting from the interaction of ergoloid (red) with estrogen receptor β-murine double minute 2 chain B (ERβ-MDM2 B).

Table 2. Estrogen receptor β (ERβ) protein with murine double minute 2 chain B (MDM2 B)

Hydrophobic interactions within 5 Å					
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
324	LEU	A	62	MET	B
324	LEU	A	67	TYR	B
325	PHE	A	61	ILE	B
325	PHE	A	62	MET	B
325	PHE	A	67	TYR	B
325	PHE	A	75	VAL	B
325	PHE	A	93	VAL	B
413	LEU	A	93	VAL	B
414	VAL	A	100	TYR	B
414	VAL	A	19	ILE	B
414	VAL	A	54	LEU	B
Protein-Protein Main Chain-Side Chain Hydrogen Bonds					
	Donor			**Acceptor**	
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
324	LEU	A	62	MET	B
414	VAL	A	96	HIS	B
412	PRO	A	18	GLN	B
412	PRO	A	18	GLN	B
324	LEU	A	72	GLN	B
324	LEU	A	72	GLN	B
497	ALA	A	73	HIS	B
Protein-Protein Side Chain-Side Chain Hydrogen Bonds					
	Donor			**Acceptor**	
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
314	LYS	A	69	GLU	B
327	GLN	A	72	GLN	B
327	GLN	A	72	GLN	B
493	GLU	A	71	GLN	B
493	GLU	A	71	GLN	B
493	GLU	A	70	LYS	B
493	GLU	A	70	LYS	B
493	GLU	A	71	GLN	B
493	GLU	A	71	GLN	B
494	MET	A	71	GLN	B
494	MET	A	71	GLN	B
494	MET	A	71	GLN	B
494	MET	A	71	GLN	B
327	GLN	A	72	GLN	B
327	GLN	A	72	GLN	B
332	GLU	A	94	LYS	B
332	GLU	A	94	LYS	B
Protein-Protein Ionic Interactions					
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
314	LYS	A	69	GLU	B
332	GLU	A	73	HIS	B
332	GLU	A	94	LYS	B
493	GLU	A	70	LYS	B
Protein-Protein Aromatic-Aromatic Interactions					
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
325	PHE	A	67	TYR	B
Protein-Protein Aromatic-Sulphur Interactions					
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
325	PHE	A	62	MET	B
ERβ B chain with MDM2 B chain					
Protein-Protein Main Chain-Side Chain Hydrogen Bonds					
	Donor			**Acceptor**	
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
364	ARG	B	18	GLN	B
Intraprotein Ionic Interactions					
Position	**Residue**	**Chain**	**Position**	**Residue**	**Chain**
365	ASP	B	97	ARG	B

LEU: leucine, MET: methionine, TYR: tyrosine, ILE: isoleucine, PHE: phenylalanine, VAL: valine, HIS: histidine, PRO: proline, GLN: gltamine, ALA: alanine, LYS: lysine, GLU: glutamic acid, ARG: arginine, ASP: aspartic acid

Ergoloid compound strengthens binding to ERβ and MDM2 complex proteins

PyRx 8.0 was used for virtual screening of Natural Product compounds as ligands with ERβ-MDM2 complex proteins, obtained ergoloid, doxorubicin, and paclitaxel compounds. Ergoloid compound, has a better bond when compared to Doxorubicin and Paclitaxel **(Table 3)**. PLIP was used to validate the amino acid residues that appear in the bond **(Table 4** and **Figure 2)**.

DISCUSSION

This docking study was conducted using ClusPro, as being able to predict protein-protein interactions is one of the major challenges facing the proteomics community. The main objective is to retrieve the three-dimensional coordinates of two independent crystallized proteins that are known to interact, and to obtain a model of the bound structure [18-19]. Based on the virtual screening, the Ergoloid compound was found to have the lowest binding affinity value of -9.7 and interacts with ERB protein complex at amino acids GLN18B; ARG186B; PRO412A; ASP378B; GLU389B **(Table 4)**. The smaller the binding affinity value, the greater the binding affinity of the ligand to its target.

Table 3. Comparison of binding affinity of ergoloid, doxorubicin, and paclitaxel.

Rank	Compound	Binding affinity	ID	Source
1	*Ergoloid (Natural Product)	-9.7	ZINC000003995616	ZINC Database
2	Doxorubicin	-9.1	ZINC000003918087	
3	Paclitaxel	-8.8	ZINC000096006020	

*selected as the best compound among the 3 candidates.

Table 4. ERβ-MDM2 B Interaction with Ergoloid

Amino acid residues	Position	Chain
GLN	18	B
ARG	386	B
PRO	412	A
ASP	378	B
GLU	389	B

GLN: Glutamine, ARG: Arginine, PRO: Proline, ASP: Aspartic acid, GLU: Glutamic acid

Conversely, the greater the binding affinity value, the weaker the target molecule and the ligand are attracted and bind to each other [20]. Docking was performed on the ERβ-MDM2 receptor, ERβ is a member of the nuclear transcription factor superfamily, encoded by the ESR2 gene (14q23.2), and consists of 530 amino acids [14]. ERβ is abundant in most normal breast epithelial cells and is estimated to be present in 20-30% of breast cancers [21]. A significant decrease in ERβ gene expression in tissues was observed in 120 patients with phase II to phase IIIA breast cancer after chemotherapy [22].

The PI3K/AKT pathway is a common pathway in tumors and is negatively regulated by homologous phosphatase enzymes and alterations on chromosome ten involving PTEN. This pathway has a role in regulating various processes such as cell growth, invasion, apoptosis, and regulation of hypoxia-associated proteins [14]. Activation of the PI3K/AKT pathway plays an important role in downregulating ERβ in breast cancer and is associated with PTEN [11]. Increased PI3-K/Akt signaling and synergistic activation of CREB-CBP coactivators induce ERβ ubiquitination and degradation processes. This process is amplified by the part of ERβ that has a negative charge. Activated Akt triggers the recruitment of the E3 ubiquitin ligase enzyme MDM2 to ERβ, which then becomes more stable thanks to interaction with CBP, resulting in ERβ polyubiquitination. By increasing PTEN levels and decreasing HER2/HER3 protooncogene signaling, Akt signaling is also decreased [14].

Murine double Minute 2 (MDM2), is a gene that functions as a negative regulator of the tumor suppressor protein, p53 (encoded by the TP53 gene) [23-24]. The p53-MDM2 protein interaction is an important target in the development of anti-tumor drugs. The interaction plays an important role in tumor cell cycle regulation, apoptosis and DNA repair directly. Activation of the p53-dependent pathway induced by internal or external cell stress signals affects the occurrence, progression and metastasis of cancer cells and prevents the proliferation of potentially carcinogenic damaged cells. In addition, as a transcription factor, various genes can be activated by p53 to drive specific tumor-related processes [24]. MDM2 proteins can bind and degrade p53, leading to decreased levels of this important tumor suppressor protein. Therefore, dysregulation of MDM2 can result in increased cell proliferation, decreased apoptosis, and ultimately, cancer progression [23]. The MDM2 protein has four functional regions: I, includes about 100 amino acid residues at the N-terminus, and in addition to binding to the p53 protein, it can also directly bind to gene promoters to activate gene transcription; II, a highly acidic region that can bind to 15 ribosomal proteins and 5 s rRNA; III, contains a zinc finger structure, which has transcription factor activity and promotes cells from G1 phase to S phase; IV, contains a CINCIN finger structure that can mediate interaction with p53 and bind to DNA or RNA to participate in cell cycle regulation and promote cell proliferation [24–25].

MDM2 also acts as an oncogene, a gene that can promote tumor development. The gene was found to be located on the long arm of chromosome 12, specifically in a region known as 12q13-14. It contains two transcriptional promoter elements named P1 and P2. The P2 promoter is dependent on p53. The MDM2 gene is expressed as different isoforms, and the full transcript of MDM2 encodes a protein of 491 amino acids that plays an important role, in that in many human cancers, the MDM2 oncogene is amplified or overexpressed, and high levels of MDM2 are associated with poor prognosis. MDM2 overexpression is strongly associated with processes linked to the presence of malignant tumors in humans and mice, including cell proliferation, DNA damage response, cell cycle regulation, and apoptosis [23].

Ergoloid or in WHO known as ergolid mesylates was introduced to the medical world in 1949 as a treatment for dementia [26]. Ergoloid is a mixture of natural products and consists of four compounds that are analogs of each other [27]. Ergoloid is used as a nootropic, similarly to piracetam to enhance brain function and these two drugs are synergistic. In addition, ergoloid is used to treat alzheimer's, stroke, and dementia [28–29]. Patients suffering from the disease

showed an improved response in overall clinical status, indicated by positive changes in mental alertness, confusion, unstable emotions, anxiety, and depression [30–31].

Ergoloid is an equiproportional of three different ergotamantrions: dihydroergocornine, dihydroergocristine, and dihydroergocryptine [32]. All of these components are produced by the fungus Claviceps purpurea and are all derivatives of the tetracyclic compound 6-methylergonovine. The derivatives of this fungus were identified to be about 350 different substances from which the ergoloid mesylate mixture component consists of hydrogenated ergot alkaloid derivatives [33].

However, this study is just a computational approach that cannot be used to draw strong and proven conclusions. Thorough investigations using in vitro, biochemical, and in vivo studies are needed to support evidence of ergoloid's role in inhibiting breast cancer cell growth.

CONCLUSIONS

Ergoloid compounds derived from natural products have potential as new breast cancer drug candidates compared to Doxorubicin and Paclitaxel drugs, because they can strengthen ERβ-MDM2 protein interactions. Further research on the effectiveness of ergoloid as a new breast cancer drug candidate needs to be carried out further through in vitro, in vivo and biochemical tests to support the results of this study.

DECLARATIONS

Competing interest
The author(s) declare no competing interest in this study.

Ethics approval and consent to participate
Not Applicable.

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