

## CYTOTOXIC ACTIVITY AND PHYSICOCHEMICAL PROPERTIES OF GENDARUSIN A-E COMPOUNDS ON ESTROGEN ALFA RECEPTORS (2JF9)

Yen Yen Ari Indrawijaya<sup>1\*</sup>, Nur Ika Octavia<sup>1</sup>, Roihatul Mutiah<sup>1</sup>, Weka Sidha Bhagawan<sup>1</sup>, Burhan Ma'arif<sup>1</sup>

<sup>1</sup>Department of Pharmacy, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim Malang, East Java, Indonesia

\*Corresponding author: yenyen.pharmacist@gmail.com

### ABSTRACT

*Estrogen Alfa (ER $\alpha$ ) is a receptor used as the main marker to identify the presence of tumors in the breast. Compounds Gendarusin A-E have anticancer activity by inhibiting the proliferation of cancer cells and inducing apoptosis. The purpose of this research is to predict the cytotoxic activity, physicochemical properties, and toxicity of the gendarusin A-E compound. The predictions of physicochemical properties were tested in compliance with the Five Lipinski Rules and the results of the ADME process (absorption, distribution, metabolism, and excretion) using the application pkCSM Online tool. Prediction of cytotoxic activity using Molegro Virtual Docker (MVD) by validating receptors and molecular docking. Cancer receptor protein used in Estrogen Alfa with PDB code 2JF9. Toxicity prediction using the Protox II Online tool. The results of this study indicate that the Gendarusin A-E compound didn't complete the Five Lipinski Rules. Gendarusin A-E compounds had activity against receptors Estrogen Alpha which is shown by the results of RMSD <2 and Gendarusin A compounds had the smallest Rerank Score of -70.9817 compared to other compounds. Gendarusin B compound had the highest LD50 1212 mg / kg and classified in grade 4.*

**Keywords:** Gendarusin A-E, Breast Cancer, Alfa Estrogen (ER  $\alpha$ ), Cytotoxic Activity, Molecular Docking

### INTRODUCTION

Breast cancer is one type of tumor that grows in breast tissue such as: mammary glands, milk ducts, fat tissue, and connective tissue in the breast [1]. Cancer prevalence is based on Basic Health Research [2] of 1.4 per 1000 occupations and is among the most common causes of death under cardiovascular disease. Chemotherapy and radiotherapy are the most effective treatment for cancer metastasis and non-metastasis [3]). The most commonly used breast cancer drug is Tamoxifen but it has side effects that cause cervical cancer in long-term use. Therefore, it is necessary to develop new drugs as breast anticancer using natural ingredients. The initial way to find out the activities of a plant quickly and does not require expensive costs is to use the method *In Silico Molecular (Docking)*. This method is used to predict whether the chemical content in a plant has anticancer activity by comparing with compounds whose effects are known as

anticancer [4]. Plants that have the potential to be anticancer are gandarusa (*Justicia gendarussa*). Gandarusa has activity as cervical anticancer on Hela cells and colon anticancer on HT-29 cells [5]. This plant contains a flavonoid compound which has been isolated, namely *gendarusin A-E*. This compound has anticancer activity which inhibits the proliferation of cancer cells by inhibiting the cell cycle and inducing apoptosis [6]. Breast cancer has receptors, one type of receptor is *Estrogen Alfa* (ER  $\alpha$ ) which is antagonistic (inhibits the growth of cancer cells). This receptor is the main marker to identify the presence of tumors in breast tissue [7]. In silico test is done by connecting the flavonoid compound structure (Gendarusin A-E) against the breast cancer receptors (*Estrogen Alfa*). Based on these descriptions, this research needs to be done to develop new drugs as breast anticancer by knowing the physicochemical properties using apps *pkCSM Online Tools* and Basic of Five Lipinski Rules, cytotoxic activity with the interaction between multiple ligands to the receptor, and toxicity using *Protox II Online Tool* to get LD50 value.

## MATERIAL AND METHOD

### Materials

*Chem Bio Draw Ultra12.0*, *Bio Chem 3D Ultra 12.0*, *Molegro Virtual Docker 6.0* (*Molegro ApS*), *pkCSM Online Tool*, and *Protox II Online Tool*. The structure of the gedarusin A-E compound and the three-dimensional structure of the receptor *Estrogen Alpha* (ER  $\alpha$ ).

### Study Design

ER  $\alpha$  PDB code: 2JF9 downloaded from the Protein Data Bank (<https://www.rcsb.org/>). The SMILES code is downloaded from the SMILES Translator Program (<https://cactus.nci.nih.gov/translate/>).

### Prediction of Physicochemical Properties and Toxicity

Prediction of Physicochemical Properties and Toxicity using SMILES code and *pkCSM Online Tool*, then predicted based on the Five Lipinski law. As for toxicity, use the *Protox II Online Tool* and classify it into the toxicity class.

### Docking and Amino Acid Analysis

Docking and amino acid analysis can be done using the Molegro Virtual Docker program, and all stages use 3D image forms. There are several steps to the docking process, namely: (1).

Download receptors from the Protein Data Bank website. The receptors that are downloaded must contain ligands. (2). Adding H atoms to the receptor (because the receptor downloaded is removed) and fixing the receptor protein downloaded, if there are errors / deficiencies of amino acids present. This stage is usually done automatically by a computer program. (3). Detection of position at the receptor, where the drug will be bound (interact). These are *cavities* in the receptor structure. (4). Put the 3D structure of the compound into the selected hole. There are several ways to put the structure of a compound in a hole, in the Molegro Virtual Docker program it is done by "*align*" which is to attach three atoms of a compound to the same three atoms on a ligand in the receptor. The selected atoms are generally the atoms in the pharmacophore group. (5). Look at the picture (*view*) the position of compounds in receptor holes (*cavities*). There are several pictures to look at the environment of compounds, including: hydrophobic picture, to see the hydrophobic environment of a compound, an electronic picture, to see the electronic environment of a compound, and a picture of the H bonding of compounds and receptors. Amino acids involved in the process of drug-receptor interactions and pharmacophore groups can be seen from the picture of the H bonds of compounds and receptors. (6). Docking compounds at the receptor, which is done automatically with the Molegro Virtual Docker program. Things that need to be considered in this process are the selection of the docking compound and the *cavity* where the drug will interact. The parameters measured in the docking process are the energy values involved, in the form of *MolDock Score*, *Rerank Score*, and *H bond*, and RMSD (*Root Mean Square Deviation*). To measure the strength of drug-receptor binding, a parameter often used is the *Rerank Score* [8].

## RESULT AND DISCUSSION

### Prediction of Physicochemical Properties and Toxicity

Prediction results of physicochemical properties using pkCSM Online Tool compound AE gendarusin none meet the criteria of Five Lipinski Law BM <500, Log P <5, Torsion 0-15, H Bond Acceptor <10, H Bond Donor <5, and PSA <140. Based on these results it can be seen that the compound Gendarusin A-E does not meet Lipinski's Law which means it has low absorption and permeability [9]. Table 1 shows the predicted results of physicochemical properties and toxicity.

Toxicity prediction results show that Gendarusin A-E compounds are classified as class 4 with susceptible LD values 500-566-1213 which means less toxic, the higher the LD value 50, the lower the toxicity. If a compound is classified as class 4 it means that it shows relatively low toxicity [10]. Based on table 1, compounds Gendarusin A, C, D, E cause toxicity to bacteria while Gendarusin B does not. Gendarusin AE does not cause toxicity to the liver and sensitivity to the skin.

### Validation of *Estrogen Alfa* Receptor

Validation is performed using *ValrogenMolegro Virtual Docker 6.0* by docking back native ligands on receptor holes resulting from the receptor validation process, namely the RMSD (Root Mean Square Deviation) value with an ideal value  $<2\text{\AA}$  [11]. The results of receptor validation are shown in table 2 and the best positions of ligands and receptors are shown in Figure 1. The results obtained from receptors *Estrogen Alfa* Chain A have an ideal RMSD value of 0.81054.

In Figure 2 shows the hydrogen bonds and steric bonds that occur between amino acids and ligands. Gendarusin A and D compounds have amino acid residues Asp 351, Glu 380, and Trp 383, gendarusin B Ala 340, Asp 351, Leu 346, Thr 347, and Leu 525, gendarusin C has only one residue, Glu 380, Gendarusin E Leu 525, Asp 351, and Glu 380. While tamoxifen as a comparison does not have active residues, but has an electrostatic interaction Asp 351.

The results of the process *docking* between the Gendarusin A-E compound and tamoxifen with the receptor *Estrogen Alpha* (ER  $\alpha$ ) with PDB code 2JF9 have a Rerank Scores of -70.9817 (gendarusin A), -68.9485 (gendarusin B), -67.1363 (gendarusin C), -67.66663 (gendarusin D), -69.6190 (gendarusin E), and -101,287 (tamoxifen) shown in Table 3.

### Discussion

The aim of this study is to predict the physicochemical properties, cytotoxic activity, and toxicity of the gendarusin AE compound against receptors *Estrogen Alfa* (ER  $\alpha$ ). Gendarusin AE compounds do not meet the Five Lipinski legal requirements in the process of predicting physicochemical properties. This means that the five compounds have low absorption and permeability. The provisions of the Five Lipinski law are molecular weights  $<500$ , Log P  $<5$ , Torsion can rotate 0-15, HBA  $<10$ , HBD  $<5$ , and PSA  $<140$  [9]. Gendarusin A-E compound has an LD50 value between 500-2000 which is 566-1213 mg / kg and is included in the toxicity class

4. Compounds classified in class IV and V show relatively low toxicity, and the higher LD50 value that means the lower toxicity [10].

Prediction of cytotoxic activity is done by docking Gendarusin AE compounds with receptors *Estrogen Alfa* (2JF9) which were previously validated by receptors by performing *redocking* molecular to select the most ideal chain. This chain selection can be seen from the RMSD results obtained in the redocking process. If the RMSD value obtained  $<2\text{\AA}$  means the value is ideal [11]. In table 2 ER  $\alpha$  (Chain A) has an RMSD value of 0.81054 which shows that chain A has an ideal RMSD compared to others.

In table 3, the *Rerank Score* of the Gendarusin compound is the smallest value compared to the others, which is -70, 6169. This indicates that the compound Gendarusin A has a high affinity and approaches the *Rerank Score* of tamoxifen as a comparison. The smaller the value of the bond energy, the more stable it is. The more stable the ligand and receptor binding, the greater the activity [12]. Gendarusin compounds A, C, D, E have hydrogen bonds with the amino acid Glu 380. In previous studies tamoxifen had a hydrogen bond with amino acids Glu 380 and Arg 394 which could increase its antagonistic activity (13). This shows that the compound Gendarusin also has the same activity as tamoxifen which inhibits the growth of cancer cells.

## CONCLUSION

Gendarusin AE compounds do not meet the Lipinski law of five and have low toxicity. Gendarusin A compound has the *lowest Rerank Score* compared to others and means the highest activity. Gendarusin compounds have almost the same activity as tamoxifen as indicated by the presence of hydrogen bonds with the amino acid Glu 380.

## ACKNOWLEDGMENTS

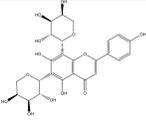
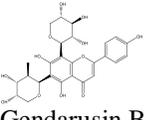
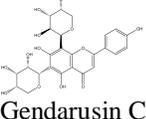
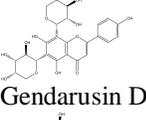
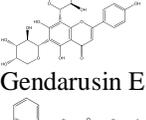
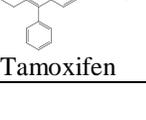
Thanks to Prof. Dr. Siswandono, Apt., MS, who has given access to the application *Molegro Virtual Docker 6.0*.

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**Table 1.** Prediction of Physical and Chemical Properties and Toxicity

Compound	TParameters of Five Lipinski Rules and Toxicity											Application of Five Lipinski Rules
	A*	B*	C*	D*	E*	F*	G*	H*	I*	J**	K**	
 Gendarusin A	534.47	- 1.1152	3	13	9	213.149	Yes	No	No	566	4	No
 Gendarusin B	532.498	0.16	3	12	8	214.720	No	No	No	1213	4	No
 Gendarusin C	534.47	- 1.1152	3	13	9	213.149	Yes	No	No	566	4	No
 Gendarusin D	534.47	- 1.1152	3	13	9	213.149	Yes	No	No	566	4	No
 Gendarusin E	534.47	- 1.1152	3	13	9	213.149	Yes	No	No	566	4	No
 Tamoxifen	371.524	5.9961	8	2	0	168.649	Yes	No	No	1190	4	No

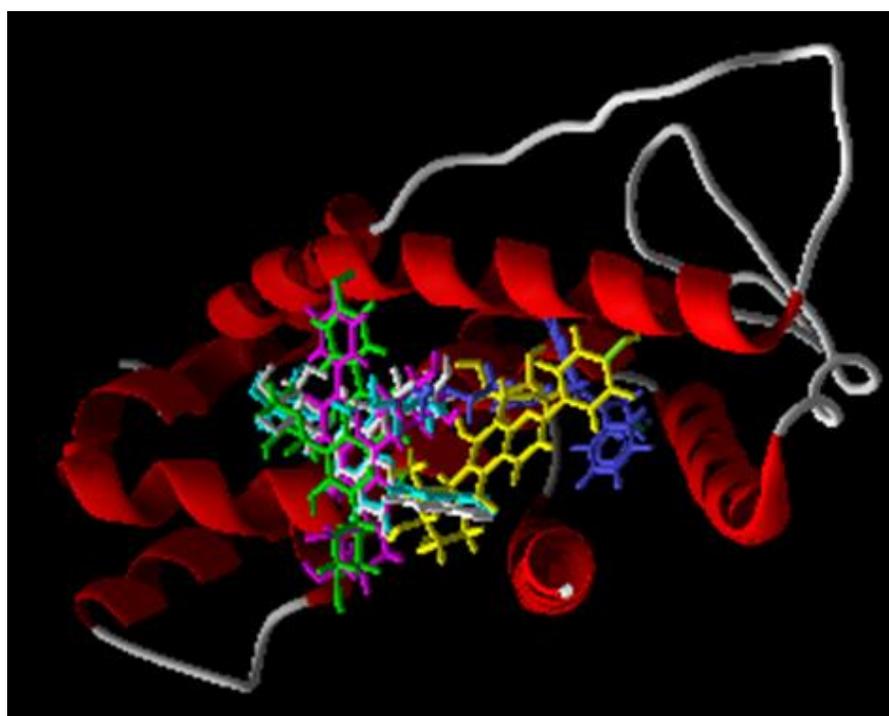
**Table 2.** Result of Receptor Validation of  $\alpha$ -Estrogen (2JF9)

Receptor (2JF9)	RMSD Score (Å)
ER $\alpha$ (Chain A)	0.81054
ER $\alpha$ (Chain B)	58.3341
ER $\alpha$ (Chain C)	58.3149

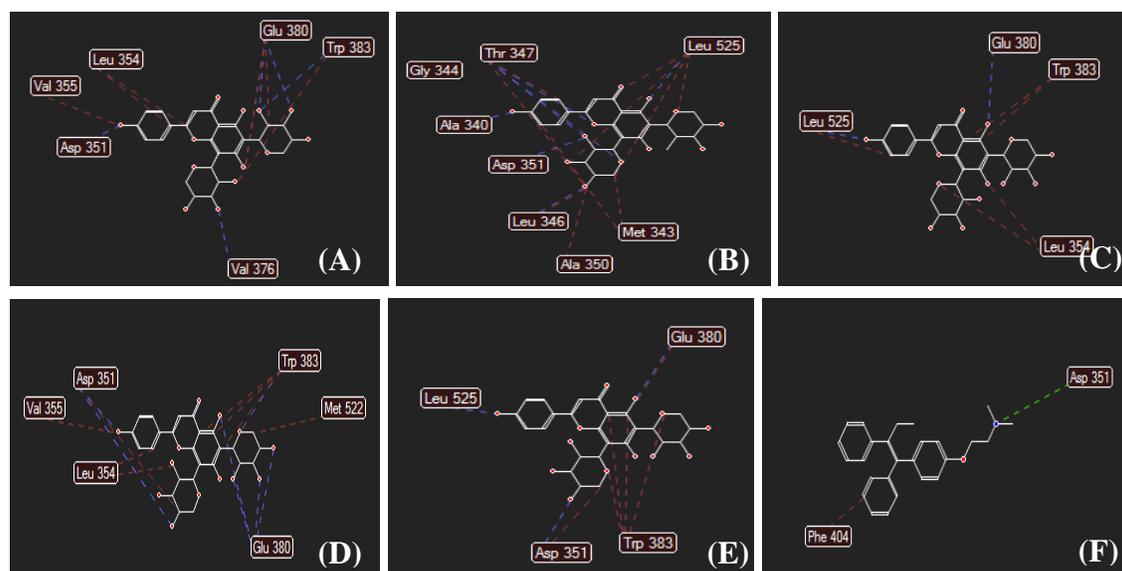
**Table 3.** Hydrogen Bonding, Steric Bonding and Rerank Score of Compounds

Compound	Hydrogen Bond and Distance (Å)	Steric Compound and Distance (Å)	Rerank Score
Gendarusin A	Asp 351 (3,23) Glu 380 (3,21 & 2,97) Trp 383 (2,67)	Val 355 (3,03) Leu 354 (2,95 & 3,13) Glu 380 (3,18 & 3,14) Trp 383 (3,07)	-70,9817
Gendarusin B	Ala 340 (2,71) Asp 351 (2,52) Leu 346 (2,72) Thr 347 (2,52) Leu 525 (3,06)	Leu 525 (2,91; 2,87; 3,18; 3,15) Thr 347 (3,01 & 2,45) Leu 346 (3,09) Ala 350 (2,87) Met 343 (3,01 & 2,45)	-68,9485
Gendarusin C	Glu 380 (3,37)	Leu 525 (3,16)	-67,1363

Gendarusin D	Asp 351 (3,22)	Leu 354 (2,86 & 3,18)	-67,6663
	Trp 383 (2,99)	Trp 383 (2,84 & 3,01)	
	Glu 380 (2,70; 2,90; 3,26; 3,36)	Val 355 (2,93)	
		Leu 354 (3,18 & 2,77)	
Gendarusin E	Leu 525 (3,02)	Asp 351 (3,02)	-69,6190
	Asp 351 (2,60)	Met 522 (3,19)	
	Glu 380 (3,30)	Trp 383 (3,16; 2,80; 3,16)	
		-	
Tamoxifen	-	Phe 404 (3,18)	-101,287



**Figure 1.** Two dimensional interaction between Gendarusin A-E and Estrogen Chain A (Red: Protein; Blue: original ligand; green: Gendarusin A; Yellow: Gendarusin B; Light blue: Gendarusin C; Purple: Gendarusin D; Gray: Gendarusin F)



**Figure 2.** The two-dimensional form of hydrogen bonds Gendarusin A (A), gendarusin B (B), gendarusin C (C), gendarusin D (D), gendarusin E (E), and tamoxifen (F) in the Estrogen Alpha receptor ( $ER\alpha$ ) with the code 2JF9 PDB. The blue lines show as hydrogen bonds, red lines as steric bonds, and green lines as electrostatic interactions.