Potential cervical anticancer activity of tomcat beetle (*Paederus fuscipes*) compounds against estrogen alpha receptor (3ert): In silico study

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Abstract. Cervical cancer is at the top of the list of women's gynecologic cancers in developing countries. Various compounds have been developed to fight cancer, but none of these compounds cause satisfactory effects. Much research has been done on anticancer drug ingredients from nature. The tomcat beetle (Paederus fuscipes) contains pederin, pseudopederin, and pederone toxins, which are suspected to have interactions and ADMET profiles against ERα receptors (3ERT) compared to the anti-cancer drugs genistein and tamoxifen. The research includes preparation, RMSD method validation, molecular docking, PreADMET, and visualization. Data were analyzed by comparing bond energy, type of bond, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity). The results obtained were the bond energies of tamoxifen -10.68 kcal/mol, genistein -7.87 kcal/mol, pederin -7.57 kcal/mol, pseudopederin -8.07 kcal/mol, and pederone -7.83 kcal/mol. The compound from the tomcat beetle with the lowest bond energy is pseudopederin. Amino acid residue interactions in P. fuscipes compounds have similarities with tamoxifen, and genistein mechanism as SERMs (selective estrogen receptor modulators). In PreADMET study, results showed that genistein, compared to tamoxifen, has more toxic effects than compounds from P. fuscipes. As conclusion, the compounds in P. fuscipes have the potential to be developed as a candidate for anticancer agents through inhibition of the alpha estrogen receptor (3ERT) based on in silico study.

1 Introduction

Cancer is one of the deadliest diseases in the world. Cervical cancer is the 2nd most common cause of cancer in women in Indonesia. An estimated 99.8 million women over 15 years old are at risk of cervical cancer, and 36,633 new cervical cancer cases occur in Indonesia. Based on the latest data, it was reported in 2021 that the death rate of people with cervical cancer touched 21,003 people [1]. Because of the adverse effects associated with current cancer treatments such as chemotherapy, radiation, immunotherapy, hormone therapy, and surgery,

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there is a need to increase the efficacy of existing treatments and/or create novel medications from natural sources, such as animal toxin-based drugs.

Animal toxin-based drugs are a well-known approach in disease treatment. Antihypertensive drugs such as Captopril (the first animal toxin-based drug approved for human use by the Food and Drug Administration [FDA] in 1981) and Enalapril (approved by the FDA in 1985) were made from bradykinin potentiating peptides (BPPs) found in Bothrops jararaca venoms [2-3]. Recenly, there are some animal toxin-based anticancer drugs have been developed. Snake venom from Bitis arietans, Cerastes gasperetti, Echis coloratus and Echis pyramidus showed anticancer properties against colorectal and breast cancer cell lines [4]. Ottoman viper (Montivipera xanthina) snake venom was cytotoxic to various cancer cells such as human prostate adenocarcinoma (LNCaP), MCF-7, human colon adenocarcinoma (HT-29) and human osteoblastic osteosarcoma (Saos-2) cells compared to non-cancerous cell line from of African green monkey kidney epithelial cells (Vero) [5].

Tomcat beetles (Paederus fuscipes) are known as kanai ants or kayap ants. [6]. Paeserus fuscipes also known as Paeserus dermatitis, due to its toxicity in human skin and caused a dermatitis symptoms. Paeserus fuscipes contained a toxin produced by pseudomonas bacteria in the aemolymph and released by the female paederus beetle [7-9]. Paederin causes a release of epidermal proteases and a loss of intercellular connection, nhibiting protein synthesis, DNA synthesis, and mitosis [10]. Pederin and its derivates pseudopederin and pederon) are an amide compound with two tetrahydrophyran rings. One individual tomcat beetle has pederin levels of approximately 0.025% of the weight of the tomcat beetle [11]. According to the toxicity of Paederin and other compounds in Paederus fuscipes, herein we investigated the potency of pederin, pseudopederin and pederon from P. fuscipes against cervical cancer cells by in silico approaches by molecular docking.

Molecular docking is a computational stage that aims to find ligand compounds that match the lowest binding energy and geometric conformation into proteins. Molecular docking is a computational method used to predict the interaction of two molecules, generating a binding model. In many drug discovery applications, docking is done between a small molecule and a macromolecule for example, protein-ligand docking. Regarding the cytotoxic potency of tomcat toxin in cervical cancer, the ERα receptor (Esterogen alpha) was select as the protein. Estrogen influences physiological and pathological processes in various tissues through its receptors. ER-α is the major estrogen receptor expressed in the cervix. Researchers found that ER-α is critical in cervical carcinogenesis in transgenic mice [12]. Previous studies have indicated that high expression of ER-α36 is associated with a more aggressive phenotype in various cancers, including breast cancer [13-14], endometrial cancer [15], gastric cancer [16], lung adenocarcinoma [17] and laryngeal cancer [18-19]. In this study, the molecular docking was done by docking the protein estrogen receptor alpha (ERα) that was downloaded from Protein Data Bank (code: 3ERT) [20] with ligand compounds pederin, pseudopederin and pederon. The potency of ERα inhibition of Pederin and its derivates were presented as the binding energy, inhibition contant and the pattern of amino-acid-ligand interaction.

Hence, the main objective of the present study is to carry out molecular docking analysis of Paederus fuscipes toxic compounds, pederin, pseudopederin and pederon against the Erα receptor followed by molecular interaction study (hydrogen bond prediction between target and drugs), drug-likeness behavior and ADMET prediction, compared to Tamoxifen and genistein, established anticancer drugs. As part of the natural anticancer drug discovery, we hope that this paper will have contribution in the third Sustainable Development Goals (SDGs), the Good health and well-being, particularly in quality and affordable medicines and vaccines.

2 Material and Methods

2.1 Tools

Hardware in the form of a Lenovo laptop with Windows 10, 64 bit operating system with AMD-A9 RADEON R5 processor specifications, 5 COMPUTE CORES 2C + 3G 3.10 GHz RAM 4.00 GB equipped with MGL-Tools®, Autodock Tools 1.5.7, Avogadro, Discovery Studio Visualizer®, Notepad++®, Chemdraw ultra, Ligplot, PyMol, Swiss PDB viewer and the sites used are Protein Data Bank, Pre-ADMET (BMDRC) PubChem, Procheck.

2.2 Materials

a) Three-dimensional structures of the test ligands, namely the compounds pederin (CID: 5381287), pseudopederin (CID: 12314693), and pederon (CID: 177799) downloaded from the PubChem website.
b) Three-dimensional structures of comparator drugs, namely Tamoxifen (CID: 2733526) and genistein (CID: 5280961) having anticancer activity used as comparators. The three-dimensional structures of the oabt were downloaded from Pubchem.
c) Receptor, The three-dimensional structure of the ERα (Estrogen alpha) Receptor was downloaded from Protein Data Bank (RSCB) the crystallography method used was X-ray with a resolution of 2Å. The code of the macromolecule is 3ERT, which was downloaded in (.pdb) format.

2.3 The Course of Research

a) Selection of Ligand
Pederin (PubChem CID: 5381287), pseudopederin (PubChem CID: 12314693) pederon (PubChem CID: 177799), Tamoxifen (PubChem CID: 2733526) and genistein (PubChem CID: 5280961) downloaded from the PubChem website and converted their file format from SDF to PDB file.
b) Selection of target receptor (ERα)
The crystal structure of the Erα receptor was downloaded on the Protein Data Bank macromolecular provider site at https://www.rcsb.org/ and saved in format (.pdb). In this study using protein identity 3ERT.
c) Preparation of Ligands and ERα Receptor
The ligands were prepared by adjusting ionization, torsion, and genistein by using the Discovery Studio Visualizer program. This procedure includes separating protein macromolecules with residues (water molecules) and natural ligands.
d) Energy Minimization
Pederin, pseudopederin, pederon and comparator compounds are energy minimized using the Avogadro program with the MMFF94 force field and then calculate energy. Then, the minimization of estrogen receptor alpha (3ERT) using the program. Swiss PDB by performing energy minimization. The macromolecules were hydrogenated (add hydrogens) with Kollman United Atoms type by using AutodockTools,. Ligand preparation was done with AutodockTools by adding partial Gasteiger charges and hydrogen (polar only).
e) Method Validation
Validation of the molecular docking method was carried out by re-docking between natural ligands of the target receptor, using Autodock Tools software. The validation

result is the Root Mean Square Deviation (RMSD) value; the binding site found and the parameters used are valid if the RMSD result is ≤ 2Å.

f) Molecular Docking
Docking was performed by docking the preprepared 3ERT protein (.pdbqt) with the test ligand and comparator drug using Autodock Tools. Grid box parameter settings are performed using Autodock 1.5.7. The grid box coordinates are determined based on the native co-crystal ligand coordinates of the receptor file used during validation, then the bonding process between the test ligand and the receptor is carried out. The magnitudes of the X, Y, and Z axes used in grid boxes setting were (40,40,40) with coordinates (30.235, -2.090, 24.385) and a grid point spacing of 0.375 Å.

g) Analysis and Visualization of Molecular Docking
Determination of the conformation of the docking ligand (the best pose) was done by selecting the conformation of the ligand that has the lowest binding affinity. The docking results with the best poses are then analyzed using Biovia Discovery Studio. Parameters analyzed included binding affinity (ΔG), inhibition constant (KI), amino acid residues, and the number of bond interactions formed. The LigPlot, Discovery Studio Visualizer, and PyMOL were used to visualize the results of the binding of the test compound to the protein by observing the presence of an interaction between the ligand and protein in 2D and PyMOL software 3D to visualize the surface area of the test ligand in the active site of 3ERT receptor.

h) ADMET properties prediction (Pre-ADMET program)
ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity. It contains the pharmacokinetic profile of active chemicals (ligands) and plays an important role in defining their pharmacodynamic properties. Considering all five substances, features such as bioavailability, brain penetration, oral absorption, carcinogenicity, and other human intestinal absorption qualities of active compounds Pre-ADMET program was used from the Pre-ADME site (BMDRC). SMILE from the structure of the selected active compounds was analyzed and stored in .pdb format.

2.4 Data Analysis

The validation of the molecular docking method was adjusted by RMSD value from re-docking analysis. The RMSD value should be ≤ 2 Å. The docking analysis was carried out by comparing the values of ΔG, Ki, molecular interactions (amino acid residues), and *binding poses* between the ligands (Pederin and its derivates) and their comparison ligands (native ligand, Tamoxifen, and Ganestein).

3 Results and Discussion

3.1 Drug likeness assessment

Prediction of physical and chemical properties of ligands was carried out based on Lipinski's (2004) rule in The Rule of Five Revolution (Lipinski's Rule of Five) where this rule helps determine whether compounds that have similarities with drugs are suitable for oral administration bioavailability if the molecular mass < 500 Dalton, LogP lipophilicity ≤5, H donor ≤5, H acceptor ≤10, molar refractivity 40 – 130 [21]. The results of the Lipinski Rule of five ligand test are presented in Table I. The results of the Lipinski analysis on the four compounds can be used for drugs by the oral route. The hydrogen bond donor and acceptor features of Pederin and its derivates were presented in Supplementary 1.

Table 1. Lipinski Rule of Five Test Result

Ligand	Molecular Weight (Dalton)	H Donor	H Acceptor	LogP	Molar Reactivity
Tamoxifen	312	0	2	-0,053101	77,145782
Genistein	270	3	5	2,419599	70,813881
Pederin	503	3	9	1,3759	128,510239
Pseudopederin	489	4	9	4,81798	132,367096
Pederon	501	2	9	1,5841	127,510445

Based on Table 1, pederin, pseudopederin and pederon compounds that have higher HBD and HBA thus the absorption of these compounds is difficult to be absorbed in the bilayer membrane compared to the drug tamoxifen which has 0 HBD and 2 HBA, but the comparison drug genistein has 3 HBD and 5 HBA, where the number of hydrogen donors (HBD) has similarities with pederin, in consequent to have similarities in physico-chemical properties in the membrane diffusion process. Log P is a parameter of hydrophobicity or lipophilicity of compounds. A larger log P value will cause the compound to be hydrophobic. The ligands pederin, pseudopederin, pederon, and the comparative drug genistein do not have a log P value below 5, tamoxifen has a negative log P value in the Lipinski prediction, which means that tamoxifen is difficult to pass through the lipid bilayer membrane and there is a possibility of interaction with polar solvents such as water. In the Lipinski test, a maximum of 2 parameters were obtained that did not fall within this range [21]. In conclusion the results obtained are declared to fulfill the Lipinski rule (RO5).

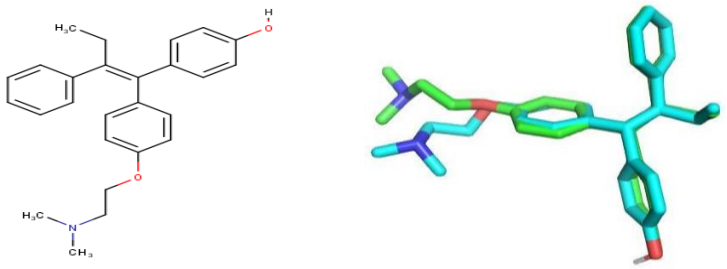
3.2 Preparation of ligands and receptor 3ERT

The x-ray structure of ERα complexed with 4-OHT (PDB ID: 3ERT) was chosen for molecular docking simulation because to its high experimental resolution (1.9 Å), R-value-free (0.262), and R-value work (0.229). The hydrophobic interaction on the 4-OHT was primarily with aromatic rings, but the butenyl group also had a positive ionizable interaction with secondary amine nitrogen. Hydrogen bond connections were created between the hydroxyl and phenoxy oxygens. The ERα's ligand-binding domain (LBD) is a hydrophobic cavity composed of amino acid residues from helices 3, 6, 7, 8, 11, and 12. A ligand's action as an agonist or antagonist is determined by helix-12 from residues 536-544 in its macromolecule (ERα). When using an antagonist, such as 4-OHT and attaches to the LBD of ERα, helix-12 closes and does not connect to the co-activator, indicating antagonistic activity due to the lack of hydrogen bond interaction with His524.16 Estradiol, an ERα agonist, interacts with His524 by a hydrogen bond [20]. The 3ERT protein preparation by Biovia Discovery Studio software before (a) and after (b) preparation was presented in Supplementary 2.

3.3 Validation method by re-docking

Validation is done by re-docking the native ligand to the target receptor, and the analysis used to evaluate the results is the RMSD (Root Mean Square Deviation) value to find a valid binding site with a tolerance of RMSD value <2 Å [22]. The superimposable of native ligand in crystallographic conformation and its redocking was presented in Figure 1. The re-docking of the receptor with the original ligand was carried out by setting the grid box based on the center point of the ligand and carried out in dimensions of 40 x 40 x 40 and obtained the

coordinates X = 30.313, Y = -1.913 and Z = 24.189 after running the re-bonding of the 3ERT receptor with the original ligand, namely 4-hydroxytamoxifen.



(a) (b)

Fig. 1. (a) Native Ligand 4-Hydroxytamoxyphene, (b) Validation properties of native ligand from docked structure (blue color) and crystallographic structure (green color)

The RMSD value obtained from re-bonding was less than 2 Å. This result indicated that the conformation obtained during re-docking is close to the conformation of the original ligand. Therefore, the docking program was valid for use in the molecular docking process [23]. This docking validation RMSD is related to Muchtaridi et al., 2018, with a value of 1.01 Å [24].

3.4 Molecular docking result

The docking process was run with the Autodock 4 program using the grid box coordinates of the validation results, namely X = 30.235, Y = -2.090, and Z = 24.385, with dimensions of 40 x 40 x 40. The binding energy and Inhibition constant of the ligands to the 3ERT receptor are presented in Table 2.

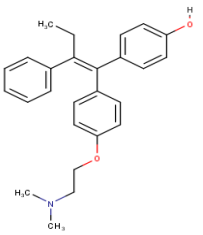
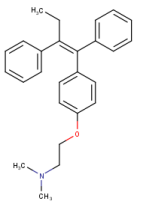
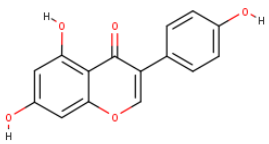
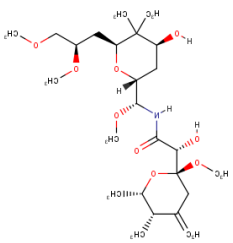
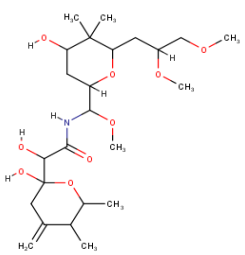
As known in receptor binding theory, the low binding energy will form more interactions between receptors and ligands because more energy is released called gibbs energy (ΔG) [25]. Therefore, among the three compounds found in tomcat beetles, pseudopederin has a gibbs energy of -8.07 kcal/mol. Pseudopederin has better stability in terms of Gibbs energy (ΔG) than pederin and pederon and the comparative anticancer drug compound genistein with Gibbs energy of -7.87 kcal/mol. However, due to the similar chemical structure with native ligand, it has the best binding energy of -10.68 kcal/mol.

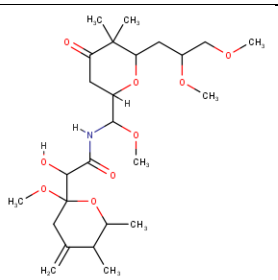
Otherwise, the Inhibition Constant (IC) is defined as the affinity to the receptor determined by the value of binding energy and inhibition constant in units of concentration. The more negative bond energy value and the smaller the inhibition constant value indicate the higher ligand affinity [26]. In this study, the results of the docking process obtained in the form of tamoxifen inhibition constant is 0.01472 µM, Genistein 1.51 µM. While the compound in the tomcat beetle pederin, pseudopederin, and pederon has an inhibition constant value of 2.81 µM, 1.22 µM, and 1.81 µM. The lowest inhibition constant (Ki) was revealed by pseudopederin. The inhibition constant of tamoxifen is the lowest at 0.01472 µM.

Although pederin and its derivates, in both the binding energy and Inhibition constant, had a lower affinity than tamoxifen, in comparison with the natural anticancer drug Ganestein, the toxic compound of Tombattle, particularly pseudopederin, had a higher affinity than Ganestein. Regarding some of the side effects of the cancer treatment by tamoxifen, such as venous thromboembolic events and endometrial cancer, in

postmenopausal women, the pederin and its derivates are promising to explore its anticancer activity by in vitro and in vivo studies. The interaction of ligands and amino acid residues of 3ERT is presented in Table IV and Figure 2.

Table 2. The Binding Energy and Inhibition Contant of Ligands to 3ERT receptor

Ligand	Chemical Structure	Binding Energy ΔG (Kkal/mol)	Inhibition Constant (μM)
4-hydroxytamoxifen		-11.04	0.0808
Tamoxifen (CID: 2733526)		-10.68	0.01472
Genistein (CID: 5280961)		-7.87	1.51
Pederin (CID: 5381287)		-7.57	2.81
Pseudopederin (CID: 12314693)		-8.07	1.22

Pederon (CID: 177799)		-7.83	1.81

Based on the results of amino acid residue interactions, pederin compounds have 20 interactions, there are 2 hydrogen bonds binding to amino acid residues, 5 hydrophobic bonds binding to the same amino acid residues as the comparator drug tamoxifen and 1 pi-sigma bond. The pseudopederin compound has interactions with 19 amino acid residues, namely 5 hydrogen bonds and 15 hydrophobic interactions which interact with the same amino acids as the drug tamoxifen. Interactions with pederon compounds have 20 interactions with amino acid residues, namely 2 hydrogen bonds, as well as 16 hydrophobic interactions that bind to the same amino acid residues as the comparison drug. Pseudopederin has the greatest amount of hydrogen bond, that supported the highest binding affinity than Pederin and Pederon. The hydroxyl and methoxyl groups seem to be the important functional group in 3ERT interaction.

Table 3. Ligand - 3ERT Receptor Amino Acid Residue Interaction

Ligand	**Type of Interaction**	**Amino Acid Residues**
4-OH Tamoxifen	Hydrogen	ARG394; HIS524
	Van der waals	GLY521; HIS524; GLY420; ILE424; GLU419; ASP351; THR347; LEU384; TRP383; LEU349; MET388
	Alkyl dan Pi-Alkyl	MET421; LEU525; LEU346; ALA350; LEU387; LEU391; PHE404; LEU428
	Pi-sulfur	MET343
	Akseptor – akseptor (unfavorable)	GLU353
Tamoxifen	Van der waals	GLY521; HIS 524; GLY420; GLU419; MET343; LEU384; THR347; ASP351; LEU354; TRP383; GLU353; ARG394; LEU349; PHE404; LEU428; ILE424
	Alkyl and Pi-Alkyl	MET421; LEU525; ALA350; LEU391; LEU387; MET388; LEU346
Pederin	Hydrogen	ASP351; THR347;
	Van der waals	LEU536; LEU354; MET357; GLU353; LEU525; LEU387; ALA350; LEU384; MET388; GLY521; ILE424; LEU391; MET421; PHE404; LEU428; LEU346; MET343
	Pi- Sigma	TRP383;

Pseudopederin	Hydrogen	ASP351; THR347; LEU346;
	Van der waals	TRP383; MET528, ALA350; LEU349; GLU353; LEU387; GLY521; ILE424; MET421; LEU391; LEU428; MET388; PHE404; MET343; LEU384; LEU525
Pederon	Hydrogen	THR347; HIS524
	Van der waals	PHE404; ILE424; MET421; LEU384; GLY420; GLY521; MET343; LEU525; ASP351; LEU354; LEU536; ALA350; MET528; LEU387; MET388; LEU346; LEU428; LEU391
Genistein	Hydrogen	GLY 521; HIS524; ARG394; GLU353; LEU346
	Van der waals	GLY420; ILE424; MET343; THR347; LEU349
	Pi-Sigma	LEU384
	Pi-Alkyl	ALA350; LEU387; LEU391
	Pi-Sulphur	MET388

As shown di Table 3, there were at least four amino acids residues that responsible in 3ERT interaction, namely GLY521; ILE424; ASP351; LEU384; LEU346; and THR347. The interaction of amino acid residues is through several types of interactions, such as hydrogen bonds, hydrophobic interactions, temporary covalent interactions (van der Waals) or a combination of the three. The interaction mode of current amino acids was related with interaction of alfa mangostin and its derivates in 3ERT receptor, particularly the hydrogen bond of Thr347 and Asp351 [24]. Moreover, according to ligand based mechanism of agonis 3ERT, the interaction with HIS524 in helix-12 is an important interaction that play important role in 3ERT activation [24]. Estradiol as a hERα agonist has a hydrogen bonding interaction with His524 [27]. Based on amino acids interaction as shown ini Table 6 and Figure 4, Pederin and Pseudopedertin were not showed an interaction with HIS524. Therefore, Pederin and pseudopederon are promising as antagonist 3ERT receptor.Futhermore, in order to develop of pederin and its derivates as the oral anticancer drugs, the pharmacokinetic prediction is important. The pharmacokinetic prediction is included of Absorption, Distribution, Metabolism, Extretion, and Toxicity that reflect the successfulness of effectiveness and safety of pederin and its derivates.

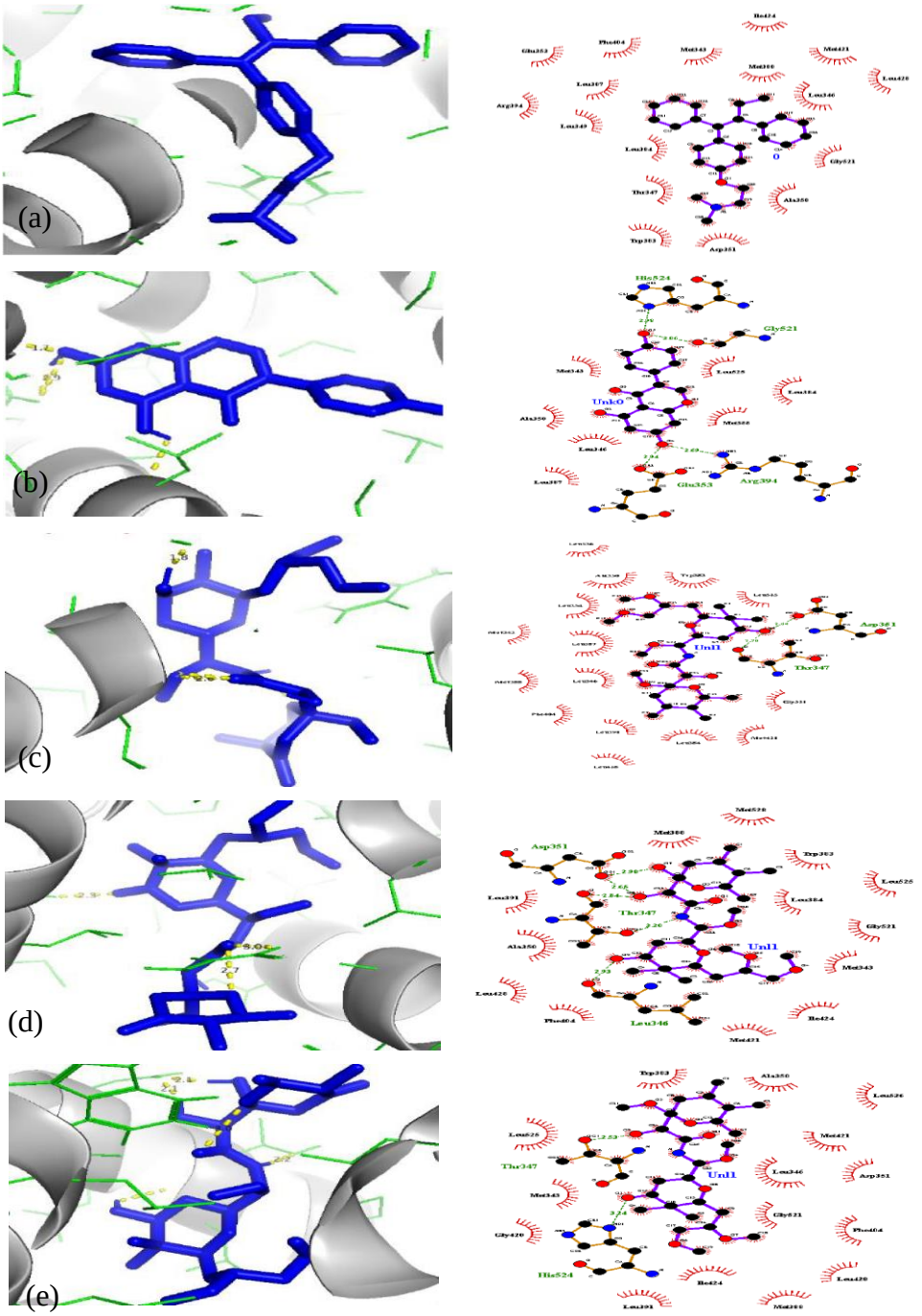


Fig. 2. 3D and 2D Visualization of 3ERT Ligan-receptor Interaction of Tamoxifen (a); Ganestein (b); Pederin (c); Pseudopederin (d); Pederon (e) by Pymol and Ligplot Program

3.5 Prediction of ADMET Profile

The aim of in silico ADME prediction is to accurately forecast the in vivo pharmacokinetic features of prospective therapeutic compounds in humans using only virtual structures. The Pre-ADMET is a tool that can characterize the pharmacokinetic profile of compounds comprehensively.

As presented in Table 4, the highest BBB value in tamoxifen is 14.1639, this value shows that the concentration of tamoxifen absorbed into the blood brain barrier is much higher than the compounds pederin, pseudopederin, pederon and the comparative drug genistein. Ma. et al through the ADME prediction guide from PreADMET divides the BBB classification, namely high absorption in the Central Nervous System (CNS) if it has a BB (Cbrain / Blood)> 2.0; medium absorption in the CNS if it has a BB (Cbrain / Blood) range of 0.1 - 2.0; low absorption in the CNS if it has a BB (Cbrain / Blood) < 0.1, tamoxifen is included in the classification of high absorption in the CNS [28].

Some chemotherapies target cancer cells in the brain therefore high BBB values are required, but treatment with various anticancer agents is often associated with adverse neurological consequences [27]. Chen found that tamoxifen is toxic to central nervous system (CNS) precursor cells at clinically relevant levels, tamoxifen causes increased cell death in the corpus callosum, and suppresses cell division in the corpus callosum, subventricular zone, and cerebral cortex of the brain [27].

Table 4. ADMET Prediction (Absorption, Distribution, Metabolism, Excretion and Toxicity Predicted by PreADMET (https://preadmet.webservice.bmdrc.org/)

No.	Compounds	Absorption		Distribution		Meetabolism	Toxicity	
		HIA (%)	BBB	PPB (%)	PgP	Inhibisi /subtrat CYP	Carcinogen-mouse	Carcinogen-rat
1	4-OHtamoxifen	100						
2	Tamoxifen	100	49,544	94,744	14,163	Non	Positive	Negative
3	Pederin	82,471	28,263	38,207	0,037	Inhibitor	Negative	Negative
4	Pseudopederin	63,905	12,357	37,507	0,053	Inhibitor	Negative	Negative
5	Pederon	84,865	33,302	40,340	0,033	Non	Negative	Positive
6	Ganestein	88,122	5,747	89,738	0,178	Non	Negative	Positive

The CaCO2 test parameter was used to determine absorption in the monolayer intestinal wall. The ADME parameter guide in PreADMET divides several classifications of CaCO2 cell permeability, namely weak permeability at PCaco-2 (nm/sec) < 4; moderate permeability at PCaco-2 (nm/sec) 4 - 70 and high permeability at PCaco-2 (nm/sec) > 70 [29]. Based on the results, all test ligand compounds pederin, pseudopederin and tamoxifen and genistein comparisons were declared to have moderate CaCO2 permeability.

Cytochrome P450 is an important enzyme in the detoxification process and is the main enzyme located in the liver. This enzyme plays an important role in the oxidation process and can facilitate the excretion of foreign organic compounds including drugs [30-31]. In this study, all of the tested compounds are metabolized by Cytochrom P450 enzyme, while Pederin and pseudopederin is potential in inhibition of this enzyme.

Distribution prediction with P-glycoprotein (Pgp) inhibition and substrate parameters is important because P-glycoprotein is one of the drug transporters that determine the absorption and release of various drugs [32]. The prediction results show that genistein, pederin, pseudopederin and pederon are nonsubstrate and noninhibitor to Pglycoprotein. Whereas tamoxifen is predicted to inhibit Pgp. Tamoxifen will cause inhibition of the role of pgp as an efflux transporter, by limiting the bioavailability of orally administered drugs by pumping them back into the lumen. This inhibits elimination of the drug into bile and urine and increases other drugs in a number of tissues such as the brain, testes, placenta and lymphocytes [33].

Protein Plasma Binding (PPB) means that only unbound (free) drugs can diffuse or transport across the cell membrane and interact with pharmacological targets. The level of plasma protein binding of drugs not only affects the action of the drug but also the effectiveness of therapy, because drugs that have been bound to proteins will be inactive and enter the elimination process [34]. Drug binding to protein is high if it has a %PPB value >90% and will be chemically weakly bound if it has a %PPB value <90.

Pederin has the lowest PPB value of 38.207903 which means it has low binding to proteins, pseudopederin compounds have a PPB value of 37.507763, pederon has a PPB value of 40.348570, genistein has a PPB value of 89.73819 and the highest is tamoxifen with a PPB of 94.744836. Pederin has a bond with plasma proteins consequently the free pederin compounds produce more effects, while tamoxifen has a bond with plasma proteins that are likely to be eliminated.

Carcinogenic toxicity tests on tamoxifen compounds have positive predictive results of carcinogens. Tamoxifen mediates its effects on endometrial cells through estrogenic and non-genomic pathways, then genomic changes arise as a carcinogen [35]. Tamoxifen can also induce enzymes that activate tamoxifen resulting in the formation of excessive metabolites and potentially toxic effects. Excessive metabolic activation will lead to carcinogenic properties of tamoxifen [36]. Pederon and Ganestein are also predicted have possibility to be carcinogen in rat.

Lastly, based on in silico study, the pederin and its derivates belongs to Paederus fuscipes had better interaction with 3ERT to Ganestein as natural anticancer drug, although had lesser interaction than Tamoxifen. However, as the part of investigating of animal-toxin based anticancer with less side effect, Pederin and its derivates is promising to be continued by in vitro and in vivo studies in further.

4 Conclusion

The docking results between ligand-receptor 3ERT obtained tamoxifen drug binding energy -10.68 kcal/mol, genistein -7.87 kcal/mol, pederin with binding energy -7.57 kcal/mol, pseudopederin with binding energy -8.07 kcal/mol and pederon -7.83 kcal/mol. The pseudopederin compound has the higher binding affinity than its derivates and Ganestein. Pederin and Pseudopederin are promising to be 3ERT antagonist due to the absent interaction with HIS524. The hydroxyl and methoxyl groups seem to be the important functional group in 3ERT interaction. Pederin and its derivates meet the lipinsky rule requirement and appropriate ADMET profile to be develop as oral dosage form, except pederon that is predicted has rat-carcinogenicity potency. However, in overall, the animal toxin drug from Tomcat bettle is potentially to be explored its in vitro and in vivo anticancer activity in further.

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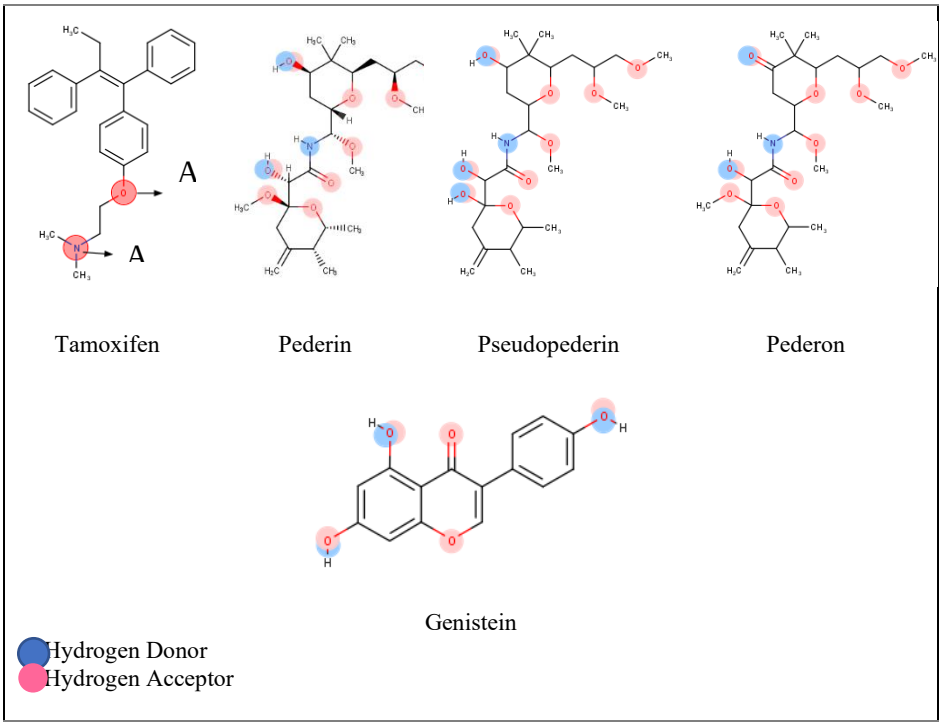
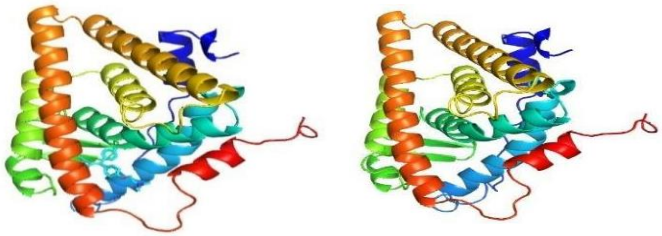
Suplementary 1. The Hydrogen Donor and Acceptor Ligand Feature of Tamoxifen, Pederin derivates and Ganistein.



Suplementary 2. The 3ERT protein preparation by Biovia Discovery Studio software before (a) and after (b) preparation.



(a) (b)