JPPIPA 10(11) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Molecular Docking: Study of Chalcone Derivatives from Boesenbergia pandurata Targeting Estrogen Receptor Alpha (ER–a) for Breast Cancer

Marsha Anggita Amelia[1], Dini Kesuma[2*], Aguslina Kirtishanti[3], I Gede Ari Sumartha[2], Maria Claudya[1]

[1] Program Pendidikan Magister Farmasi Industri, Fakultas Farmasi, Universitas Surabaya, Surabaya, Indonesia.
[2] Departemen Kimia Farmasi, Fakultas Farmasi, Universitas Surabaya, Surabaya, Indonesia.
[2] Departemen Farmasi Klinis Komunitas, Fakultas Farmasi, Universitas Surabaya, Surabaya, Indonesia.

Received: August 04, 2024
Revised: October 09, 2024
Accepted: November 25, 2024
Published: November 30, 2024

Corresponding Author:
Dini Kesuma dinikesuma1302@gmail.com

DOI: 10.29303/jppipa.v10i11.8734

Abstract: The increasing number of cancer patients and the challenge of multidrug resistance (MDR) demand more effective drugs, which can be developed by modifying compounds derived from natural resources, such as the flavonoid-rich temu kunci rhizome (*Boesenbergia pandurata* (Roxb.) Schlecht.). This study aims to predict the cytotoxicity and toxicity of 20 Pinostrobin derivatives and 19 Chalcone derivatives as potential anticancer candidates. Estrogen receptor alpha (ER-α), a validated cancer therapy target, was used for molecular docking in in silico tests using Molecular Graphics Laboratory (MGL) Tools (including, AutoDock Vina, AutoDock Tools 4.1, and Python 2.5.2) and PyRx Program. Toxicity was predicted using the pkCSM program and Protox online tool. The docking process involved binding the compounds to ER-α (PDB IDs 6CHZ and 3ERT), with the binding energy indicating activity; lower binding energy values suggest greater cytotoxic potential and stronger ligand-receptor interactions. The results showed that Chalcone derivatives from temu kunci exhibited lower toxicity and higher cytotoxic activity compared to Pinostrobin derivatives and the reference compound, Tamoxifen (TAM). Notably, Bis-3-chlorobenzyloxychalcone and Bis-2-chlorobenzyloxychalcone demonstrated the highest predicted cytotoxic activity. In conclusion, Chalcone derivatives are promising candidates for further development as more effective anticancer drugs, especially those that outperform Tamoxifen. These findings highlight the potential of natural compounds, particularly Chalcone derivatives, in combating cancer while addressing the growing challenge of MDR in clinical treatments.

Keywords: Chalcone; Cytotoxic Activity; Molecular Docking; Toxicity; Temu Kunci Rhizome

Introduction

Drug design is an advanced approach to improving existing drugs by enhancing their efficacy and minimizing side effects through molecular manipulation. This process typically involves synthesizing derivatives of parent compounds, identifying their structures, and testing their biological activity (Siswandono, 2016). Changes in the structure of a compound will alter its physicochemical properties, such as lipophilicity, electronic characteristic, and steric properties, in which these alterations in physicochemical properties will ultimately lead to changes in the biological activity of a compound (Hardjono et al., 2016).

Technological advancements enable more precise drug design through molecular modeling techniques

How to Cite:
Amelia, M. A., Kesuma, D., Kirtishanti, A., Sumartha, I. G. A., & Claudya, M. (2024). Molecular Docking: Study of Chalcone Derivatives from Boesenbergia pandurata Targeting Estrogen Receptor Alpha (ER–a) for Breast Cancer. *Jurnal Penelitian Pendidikan IPA*, *10*(11), 8376–8386. https://doi.org/10.29303/jppipa.v10i11.8734

(Schlick, 2010), so that predicting the physicochemical properties, biological activity, and toxicity of compounds before synthesis is a viable approach to take prior to the synthesis process. Known as in silico testing, molecular modeling plays a crucial role in medicinal chemistry by aiding in the design, discovery, and optimization of bioactive compounds in the drug development process (Hinchliffe, 2008; Siswandono, 2016). The interaction between a compound and a target protein is represented by a docking score, or called binding energy, which indicates the binding affinity of the compound to the target protein, the affinity value of a bond is not linear or inversely proportional to the bond strength of the compound and protein, therefore, a higher bond energy value indicates a lower affinity between the receptor and the ligand (Saputri et al., 2016).

Given the growing number of cancer patients and the rise of multidrug resistance (MDR), the need for more effective anticancer drugs is critical (D. Kesuma et al., 2022; Pratama et al., 2022). Breast cancer remains one of the most prevalent cancers, accounting for millions of cases worldwide, including in Indonesia. Based on Global Burden Cancer data (2020), it is estimated that the incidence and death rate of cancer worldwide reaches 19.3 million new cancer cases. Specifically, breast cancer cases account for an estimated 2.3 million new cases (11.7%) (Sung et al., 2021). In Indonesia, there are 396,914 new cancer cases, where breast cancer ranks first with a total of 65,858 (16.6%) and the death rate is reported to reach 22,430 (9.6%) (The Global Cancer Observatory, 2021). One major limitation of current treatments, such as Tamoxifen, is the potential for resistance after prolonged use, along with side effects like an increased risk of endometrial cancer (Ali et al., 2016). This has led researchers to seek alternative treatments that are both effective and environmentally sustainable, such as to preserve the environment and leverage biodiversity through a back-to-nature approach. Thus, this study focuses on developing anticancer drugs from natural resources, particularly through the synthesis of Chalcone derivatives from Pinostrobin, a compound found in temu kunci rhizomes (*Boesenbergia pandurata* (Roxb.) Schlecht.).

The fingerroot rhizomes (*Boesenbergia pandurata* (Roxb.) Schlecht.) known as 'Temu Kunci' in Indonesia, is rich in various compounds, including primary and secondary metabolite compounds like flavonoids and flavonoid derivatives, such as chalcones, flavanones, and flavones, offers promising natural compounds for drug development (Yap Li Ching et al., 2007). According to the Indonesian Herbal Pharmacopoeia (2017), the identity compound or marker compound of temu kunci is Pinostrobin (5-hydroxy-7-methoxyflavanone), which is a flavonoid compound in temu kunci (Departemen Kesehatan Republik Indonesia, 2017). Structural modification involving the synthesis of a series of derivatives of the parent compound is a systematic elaboration process aimed at further developing existing drugs to obtain more effective new drugs (Siswandono, 2016). In this study, Pinostrobin derivatives were synthesized into Chalcone compounds, aiming to explore their potential as anticancer agents. Given the scarcity of naturally derived Chalcone compounds, synthetic methods were used to create derivatives for testing. Molecular hybridization techniques from previous research have shown that modifications can enhance the activity of these compounds, making them potential candidates for breast cancer treatment.

In silico tests were conducted on 20 Pinostrobin derivatives and 19 Chalcone derivatives to predict their cytotoxic activity against estrogen receptor alpha (ER-α), a validated drug target in breast cancer therapy. There are approximately 70% of breast cancer patients who are hormone dependent with tumor cells that can express estrogen receptors (ER), known as luminal A and B. Estrogen is the main signal that plays an important role in the growth and development of tumors in breast cancer patients. The primary cellular responses of estrogen are mediated by nuclear ERα, ERβ, and G-protein-coupled estrogen receptor (GPER, also known as GPR30). ER-α is considered as a receptor that is most involved in breast cancer development, making it a crucial target for breast cancer treatment (Comşa et al., 2015). These tests measured the binding energy between the compounds and ER-α (PDB IDs 6CHZ and 3ERT). Compounds with lower binding energy values were predicted to have higher cytotoxic potential. This study seeks to identify the most promising Pinostrobin and Chalcone derivatives for further development as effective anticancer drugs, with a particular focus on overcoming resistance in breast cancer treatments.

Method

Materials

The device utilized in this study comprises hardware in the form of a set of desktop computers equipped with CPU Intel® Core (TM) i7-9700F CPU @ 3.00GHz, 16384MB of RAM, and the Windows 10 Education 64-bit operating system, provided by Universitas Surabaya.

The research materials used were the three-dimensional (3D) structure of the Chalcone compound and 18 of its derivative compounds in (.mol2) format (downloaded from MarvinSketch software), Molecular Graphics Laboratory (MGL) Tools (including AutoDock Vina, AutoDock Tools 4.1, and Python 2.5.2), BIOVIA Discovery Studio Visualizer (molecular visualization).

The target receptors were obtained from the PDB Database (https://www.rcsb.org/) in .pdb format, with PDB IDs 6CHZ and 3ERT (Human Estrogen Receptor Alpha), and Tamoxifen (TAM) was used as the positive control.

Ligand preparation

The ligands in this study were chalcone compounds and their derivatives (Table 1) which were prepared using the MarvinSketch and AutoDockTools 1.5.6 programs. The ligand structure image was created using the MarvinSketch program in (.mol2) format, followed by ligand preparation using the AutoDockTools 1.5.6 program, and Grid Box settings focused on the ligand.

Receptor preparation

The PDB IDs format of human ER–a (6CHZ and 3ERT) in complex with Tamoxifen (TAM) was obtained from the Protein Data Bank and downloaded via the RCSB macromolecular biology structural information portal (http://www.rcsb.org/pdb/). Each protein has a resolution of 1,90Å and 1,68Å, and was then prepared using AutoDockTools 1.5.6. Prior to testing, orientation adjustments were made to achieve the lowest RMSD value, namely under 2Å within the grid box parameter.

Molecular docking

The docking process was performed with Molecular Graphics Laboratory (MGL) Tools (including AutoDock Vina, AutoDock Tools 4.1, and Python 2.5.2) by entering commands into the Command Prompt. Additionally, the *PyRx* Program, downloaded from https://pyrx.sourceforge.io/, was used. Visualization of the docking results was done with BIOVIA Discovery Studio Visualizer (DSV), allowing the interactions between the receptor and the ligand to be viewed in both 2D and 3D using DSV.

Prediction of physicochemical properties and toxicity of compounds

The prediction of compound activity was done through the PASS Online (Prediction of Activity Spectra for Substance) website of https://www.way2drug.com/PASSOnline/index.php, the prediction of toxicity and bioavailability was done through pkCSM (Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signature) website of https://biosig.lab.uq.edu.au/pkcsm/, the analysis of the lipophilic properties of compounds based on Lipinski's Rule of Five was on the website of http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp, and to see the toxic dose and toxicity class was by using the Protox online tools on the website of https://tox.charite.de/protox3/#.

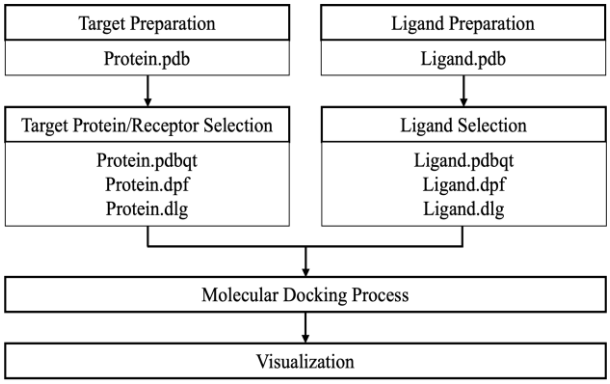

Figure 1. The Schematic flowchart

Result and Discussion

Theoretically, pinostrobin compounds can be obtained through the isolation of temu kunci (*Boesenbergia pandurata* (Roxb.) Schlecht.) plants, which can be subsequently synthesized into chalcone derivatives by adding an excess of strong base. This synthesis process offers the potential to develop new compounds with enhanced biological activity, contributing to the search for more effective therapeutic agents. By utilizing natural resources like temu kunci, it is possible to explore environmentally sustainable methods for drug discovery and development. The strategic transformation through chemical reactions aims to explore the potential of chalcone derivatives in offering better therapeutic efficacy compared to their parent compound.

Mass, et al. (2022) synthesized chalcone compounds into hybrid compounds (Chalcone-Dihydropyrimidinone) and discovered through structural analysis process that there were 10 derivative compounds from the hybrid compound. From this study, an in-silico test was performed which showed that 3 hybrid compounds, namely compounds 9d, 9g, and 9h, were compounds that had more active potential compared to the parent compound and demonstrating the effectiveness of molecular hybridization against cytotoxicity. Compounds 9d, 9g, and particularly 9h showed high selectivity for breast cancer cells (MCF-7) related to human keratinocyte (HaCaT). Molecular docking calculations for hybrid compounds 9d, 9g, and 9h positioned at the ERα active site potentially act as antagonistic molecules that could disrupt or inhibit the proliferation process of MCF-7 cells, strengthening the observed potency and selectivity for this tumor cell line (Mass et al., 2022).

Figure 2 and Table 1 display the structures of the Pinostrobin compound, Chalcone derivative

compounds, and the reference compound Tamoxifen (TAM). The Pinostrobin compound.

Prediction of physicochemical properties and toxicity of compounds

In 1997, Lipinski et al. analyzed 2,245 drugs in the World Drugs Index database, which then concluded that a compound would be difficult to absorb and have low permeability if it had a molecular weight exceeding 500Da; a log value of the octanol/water partition coefficient (log P) over +5; the hydrogen bond donors (HBD) greater than 5, indicated by the number of O-H and N-H groups; and the hydrogen bond acceptors (HBA) greater than 10, indicated by the number of O and N atoms (Lipinski et al., 1997).

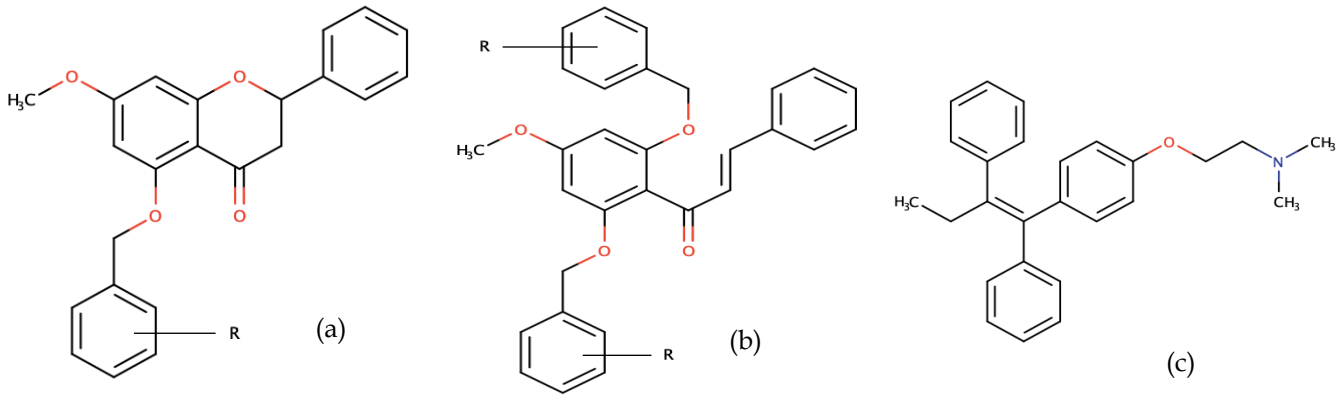

Figure 2. Pinostrobin compound (a); Chalcone derivative compound (b); and Reference compound Tamoxifen (c)

Table 1. Chemical structure of Pinostrobin and Chalcone derivative compound

No. Compound	Position	R	Compound Name
1.	–	–	5-O-benzylpinostrobin
2.	4	Cl	5-O-(4-chloro-benzyl)pinostrobin
3.	3,4	2 Cl	5-O-(3,4-dichloro-benzyl)pinostrobin
4.	3	CF_3	5-O-(3-trifluoromethyl-4-chloro-benzyl)pinostrobin
	4	Cl	
5.	3	CF_3	5-O-(3-trifluoromethyl-4-nitro-benzyl)pinostrobin
	4	NO_2	
6.	4	CF_3	5-O-(4-trifluoromethyl-benzyl)pinostrobin
7.	4	Br	5-O-(4-bromo-benzyl)pinostrobin
8.	4	I	5-O-(4-iodo-benzyl)pinostrobin
9.	2,4	2 Cl	5-O-(2,4-dichloro-benzyl)pinostrobin
10.	4	NO_2	5-O-(4-nitro-benzyl)pinostrobin
11.	3	Cl	5-O-(3-chloro-benzyl)pinostrobin
12.	2	Cl	5-O-(2-chloro-benzyl)pinostrobin
13.	2	OCH_3	5-O-(2-methoxy-benzyl)pinostrobin
14.	4	F	5-O-(4-fluoro-benzyl)pinostrobin
15.	4	$C(CH_3)$	5-O-(4-tert-butyl-benzyl)pinostrobin
16.	3	CF_3	5-O-(3-trifluorometil-benzyl)pinostrobin
17.	3,5	2 Cl	5-O-(3,5-dichloro-benzyl)pinostrobin
18.	4	CH_3	5-O-(4-methoxy-benzyl)pinostrobin
19.	4	NH_2	5-O-(4-amino-benzyl)pinostrobin
20.	4	OH	5-O-(4-hydroxy-benzyl)pinostrobin
21.	–	–	2-6-dibenzyloxykalkon
22.	4	Cl	Bis-4-chlorobenzyloxychalcone
23.	3,4	Cl	Bis-3,4-dichloro-benzyloxychalcone
24.	3	CF_3	Bis-3-trifluoromethyl-4-chloro-benzyloxychalcone
	4	Cl	
25.	3	CF_3	Bis-3-trifluoromethyl-4-nitro-benzyloxychalcone
	4	NO_2	
26.	4	CF_3	Bis-4-trifluoromethylbenzyloxychalcone

No. Compound	Position	R	Compound Name
27.	4	Br	Bis-4-bromobenzyloxychalcone
28.	4	I	Bis-4-iodobenzyloxychalcone
29.	2,4	2 Cl	Bis-2,4-dichlorobenzyloxychalcone
30.	4	NO_2	Bis-4-nitrobenzyloxychalcone
31.	3	Cl	Bis-3-chlorobenzyloxychalcone
32.	2	Cl	Bis-2-chlorobenzyloxychalcone
33.	2	CH_3	Bis-2-methoxybenzyloxychalcone
34.	4	F	Bis-4-fluorobenzyloxychalcone
35.	4	$C(CH_3)$	Bis-4-tertbutyl-benzyloxychalcone
36.	3	CF_3	Bis-3-trifluoromethyl-benzyloxychalcone
37.	4	CH_3	Bis-4-methoxy-benzyloxychalcone
38.	4	NH_2	Bis-4-amino-benzyloxychalcone
39.	4	OH	Bis-4-hydroxy-benzyloxychalcone
40.		Reference Compounds	Tamoxifen

The requirements of the analysis are known as Lipinski's Rule of Five because all values are multiples of five. Based on table 2, it can be analyzed that not all Pinostrobin and Chalcone derivative compounds examined meet the requirements of Lipinski's Rule of Five. This is demonstrated by the molecular weight of the compound, which exceeds 500 Da but remains close to it at 519.424 Da, as well as the LogP value, which is greater than 5. However, all compounds meet the requirements for hydrogen bond donors and acceptors. This is generally expected, as the predicted compounds are natural substances with bulky or large groups. Furthermore, the LogP value of the Chalcone derivatives is higher than 5 due to the addition of a benzene ring, which affects the LogP value. As is known, Pinostrobin belongs to the flavonoid subgroup flavanone, consisting of a C1–C2–C3 backbone. When Chalcone compounds are successfully synthesized from Pinostrobin, the number of benzene rings increases, resulting in higher lipophilicity for the Chalcone derivatives, making them more difficult to absorb and/or reducing their ability to penetrate membranes (Faleye et al., 2024).

According to Kumar and Pandey in their review article "Chemistry and Biological Activities of Flavonoids: An Overview," flavonoids have garnered significant attention for their health benefits, including antioxidant, anti-inflammatory, anticancer, and antiviral activities, with their bioavailability and effects largely determined by their structure and functional groups. Their bioavailability and biological effects depend on structure, hydroxyl groups, and functional substitutions. Main dietary sources include fruits, vegetables, tea, and wine. Recent research focuses on flavonoids' roles in human health, while microbial biotechnology enables cost-effective production for pharmaceutical use (Kumar & Pandey, 2013).

In silico prediction of activity and toxicity

The results of the in silico docking test between Pinostrobin and Chalcone derivative compounds with the target Estrogen Alpha receptor (PDB IDs 6CHZ and 3ERT) are presented in **Table 3**. This table shows the Binding Energy values of Pinostrobin and Chalcone derivative compounds, which can be used to predict the activity of these compounds.

In the PBD ID 6CHZ, various binding energy values were obtained, though several compounds exhibited smaller values compared to the parent compounds of Pinostrobin and Chalcone, i.e. in compounds number 5, 10, 11, 12, 13, 15, 17, 20, 22, 23, 31, 33, 34, 35, 36, 37, 38, and 39, the best binding energy value was in compound number 31 namely Bis-3-chlorobenzyloxychalcone compound (binding energy value = -11.92 kcal/mol), smaller when compared to the parent compound Pinostrobin (binding energy value = -10.07 kcal/mol), the parent compound of Chalcone (binding energy value = -11.35 kcal/mol), and smaller than the reference compound Tamoxifen (binding energy value = -10.00 kcal/mol).

Table 2. In silico prediction of the values of physicochemical parameters of Pinostroin derivatives, Chalcone, and reference compounds Tamoxifen using the pkCSM online tool. MW = Molecular Weight; LogP = Logarithm of Octanol/Water Partition Coefficient; RB = Rotation Bonds; HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors; PSA = Polar Surface Activity.

No. Compound	MW	Log P	RB	HBA	HBD	PSA (A^2)
1.	270.284	3.1073	2	4	1	116.125
2.	394.854	5.6341	5	4	0	168.170
3.	429.299	6.2875	5	4	0	178.473
4.	462.851	6.6529	5	4	0	187.031
5.	473.403	5.9077	6	6	0	191.381
6.	428.406	5.9995	5	4	0	176.728
7.	439.305	5.7432	5	4	0	171.734
8.	486.305	5.5853	5	4	0	177.128
9.	429.299	6.2875	5	4	0	178.473
10.	405.406	4.8889	6	6	0	172.520
11.	172.520	5.6341	5	4	0	168.17
12.	394.854	5.6341	5	4	0	168.17
13.	390.435	4.9893	6	5	0	169.345
14.	378.399	5.1198	5	4	0	162.032
15.	416.517	6.2782	5	4	0	183.326
16.	428.406	5.9995	5	4	0	176.728
17.	429.299	6.2875	5	4	0	178.473
18.	374.436	5.28912	5	4	0	164.232
19.	375.424	4.5629	5	5	1	163.207
20.	376.408	4.6863	5	5	1	162.661
21.	450.534	6.7493	10	4	0	199.605
22.	519.424	8.0561	10	4	0	220.211
23.	588.314	9.3629	10	4	0	240.818
24.	655.418	10.0937	10	4	0	257.935
25.	676.522	8.6033	12	8	0	266.634
26.	586.528	8.7869	10	4	0	237.328
27.	608326	8.2743	10	4	0	227.34
28.	702.326	7.9585	10	4	0	238.129
29.	588.314	9.3629	10	4	0	240.818
30.	540.528	6.5657	12	8	0	228.911
31.	519.424	8.0561	10	4	0	220.211
32.	519.424	8.0561	10	4	0	220.211
33.	510.586	6.7665	12	6	0	222.562
34.	486.514	7.0275	10	4	0	207.936
35.	562.75	9.3443	10	4	0	250.524
36.	586.528	8.7869	10	4	0	237.328
37.	450.534	6.7493	10	4	0	199.605
38.	519.424	8.0561	10	4	0	220.211
39.	588.314	9.3629	10	4	0	240.818
40.	655.418	10.0937	10	4	0	257.935

In the PDB ID 3ERT, various binding energy values were also obtained, all Pinostrobin derivative compounds have binding energy values that are smaller than the parent compound of Pinostrobin, but in the Chalcone derivatives there are only a few smaller compounds, i.e. in compounds number 23, 28, 29, 31, 32, and 35, the best binding energy value is in compound number 32, namely the Bis-2-chlorobenzyloxychalcone compound (binding energy value = -11.84 kcal/mol), smaller when compared to the parent compound of Pinostrobin (binding energy value = -8.77 kcal/mol), the parent compound of Chalcone (binding energy value = -10.96 kcal/mol), and smaller than the reference compound Tamoxifen (binding energy value = -11.05 kcal/mol).

Table 3. In silico prediction of anticancer activity and toxicity against ER–a receptors of Pinostrobin derivatives, Chalcones, and reference compounds using AutoDock Vina and PyRx (*) as well as pkCSM (**) and Protox online tools (***)

No. Compound	Activity						Toxicity
	Binding Energy* (PDB ID 6CHZ)	Binding Energy* (PDB ID 3ERT)	Ames Toxicity**	Hepa-totoxicity**	Skin Sensitization**	LD_{50} Acute**	Class***
1.	-10.07	-8.77	No	No	No	2147	5
2.	-9.23	-9.34	No	No	No	2507	5
3.	-9.8	-10	No	Yes	No	2635	5
4.	-9.69	-9.66	No	Yes	No	2816	5
5.	-11.67	-11.3	No	Yes	No	2553	5
6.	-8.85	-8.95	No	Yes	No	2721	5
7.	-9.45	-9.45	No	Yes	No	2516	5
8.	-9.87	-9.31	No	No	No	2530	5
9.	-9.63	-9.54	No	No	No	2636	5
10.	-11.44	-11.51	Yes	Yes	No	2480	5
11.	-10.56	-9.51	No	No	No	2510	5
12.	-10.66	-9.27	No	No	No	2542	5
13.	-10.29	-8.92	Yes	No	No	2675	5
14.	-10	-8.78	No	No	No	2676	5
15.	-10.44	-9.47	No	Yes	No	2492	5
16.	-10.1	-9.26	No	Yes	No	2725	5
17.	-10.86	-8.91	No	Yes	No	2606	5
18.	-10.49	-9.37	No	No	No	2446	5
19.	-10.1	-9.49	No	Yes	No	2725	5
20.	-10.79	-9.9	No	Yes	No	2606	5
21.	-11.35	-10.96	No	Yes	No	2048	5
22.	-10.68	-10.7	No	No	No	2198	5
23.	-11.09	-11.39	No	No	No	2312	5
24.	-10.7	-10.08	No	Yes	No	2500	5
25.	-9.52	-8.58	Yes	No	No	2543	5
26.	-9.86	-9.32	No	Yes	No	2400	5
27.	-11.63	-10.88	No	No	No	2198	5
28.	-11.16	-11	No	No	No	2224	5
29.	-11.15	-11.95	No	No	No	2373	5
30.	-9.49	-8.9	Yes	Yes	No	2518	5
31.	-11.92	-11.1	No	No	No	2211	5
32.	-11.54	-11.84	No	No	No	2235	5
33.	-10.54	-10.74	No	No	No	2363	5
34.	-10.34	-10.43	No	Yes	No	2395	5
35.	-10.98	-11.16	No	Yes	No	1952	4
36.	-10.61	-10.36	No	Yes	No	2382	5
37.	-10.77	-9.88	No	No	No	2754	5
38.	-9.56	-9.48	No	No	No	2346	5
39.	-10.56	-9.79	No	Yes	No	3210	5
40.	-10	-11.05	No	Yes	No	2671	5

The Bis-3-chlorobenzyloxychalcone and Bis-2-chlorobenzyloxychalcone compounds were chosen because they do not cause hepatotoxic effects. To determine the toxicity of a compound can be performed using the Ames Toxicity test, which is a widely used method to assess the mutagenic potential of a compound using bacteria. Positive test results indicate that the compound is mutagenic, which means it can act as a carcinogen. Based on Table 3, almost all Pinostrobin and Chalcone derivative compounds, along with reference compounds, are predicted not to cause mutagenic effects, except for compounds number 10, 13, 25, and 30 whose Ames Toxicity test results showed positive results.

Moreover, according to Table 3, all of these derivative compounds are predicted not to cause skin sensitization. The Protox online tool, based on the Globally Harmonized System (GHS), can be used for in silico testing and classification of oral toxicity (LD50) in rodents for Pinostrobin and Chalcone derivative compounds. LD50 is the dosage of compound given to a group of test animals that is expected to cause 50% death. If based on Table 3, almost all Pinostrobin and Chalcone derivative compounds are predicted to have

LD50 values in rodents ranging from 1952 to 3210 mg/kg, classifying them in toxicity class 5, except for compound 35 which has an LD50 value of 1952 mg/kg, placing it in toxicity class 4. If the dose of a compound is included in toxicity class 5, it is interpreted to have a low acute toxicity effect, while those in class 4 is interpreted to have relatively low toxicity.

Furthermore, considerations of the data obtained were made, and it showed that compounds number 31 and 32, specifically Bis-3-chlorobenzyloxychalcone (binding energy value = -11.92 kcal/mol) and Bis-2-chlorobenzyloxychalcone (binding energy value = -11.84 kcal/mol), were predicted to have the highest cytotoxic activity while being non-toxic, both compounds also did not exhibit hepatotoxic or other toxic effects. Therefore, this allows for the synthesis of the two selected compounds to proceed. Figure 3 and 4 shows a 3D image of the target estrogen receptor alpha (ER–α) PDB ID 6CHZ with the Bis-3-chlorobenzyloxychalcone and PBD ID 3ERT with the Bis-2-chlorobenzyloxychalcone, Pinostrobin and Chalcone compounds, as well as the reference compound Tamoxifen (TAM).

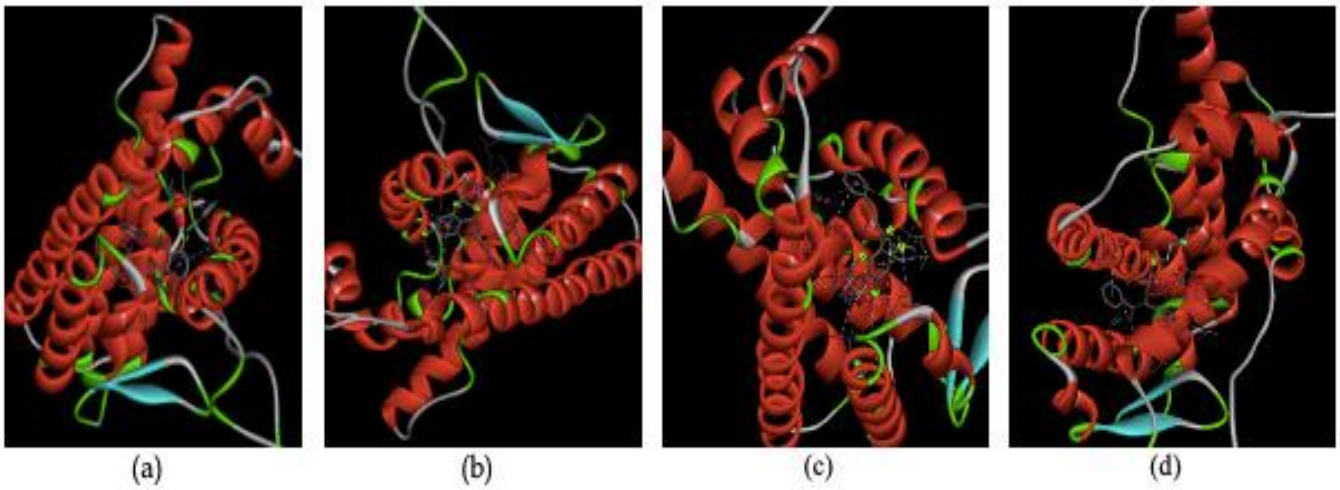

Figure 3. 3D target receptor estrogen alpha (ER–α) PDB ID 6CHZ with ligand compound Bis-3-chlorobenzyloxychalcone (a); Pinostrobin compound (b); Chalcone compound (c); and reference compound Tamoxifen (d)

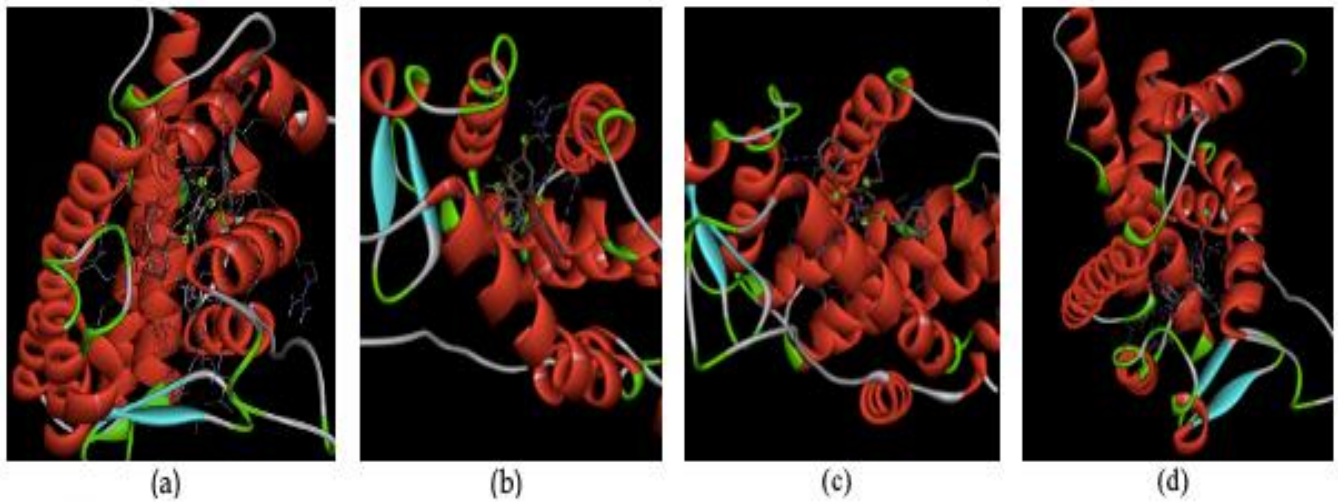

Figure 4. 3D target receptor estrogen alpha (ER–α) PDB ID 3ERT with ligand compound Bis-2-chlorobenzyloxychalcone (a); Pinostrobin compound (b); Chalcone compound (c); and reference compound Tamoxifen (d)

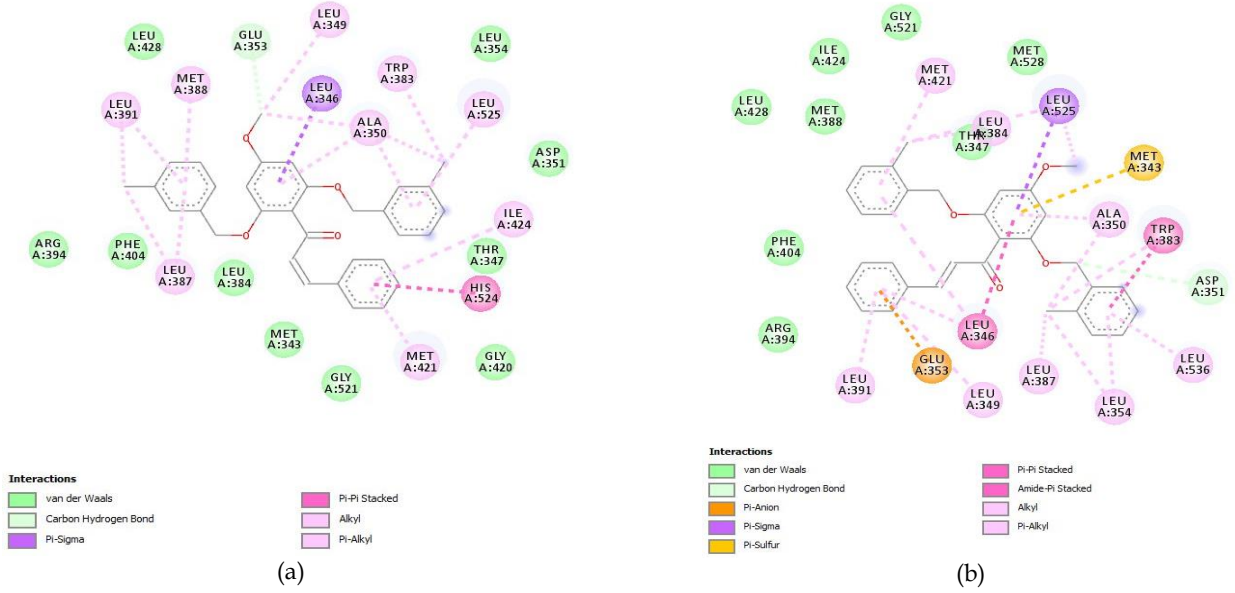

Figure 5. 2D binding site on the target estrogen receptor alpha (ER–a) PDB ID 6CHZ with the ligand compound Bis-3-chlorobenzyloxychalcone (a) and the target estrogen receptor alpha (ER–a) PDB ID 3ERT with the ligand compound Bis-2-chlorobenzyloxychalcone (b)

Table 4. The target amino acids of the estrogen receptor alpha (ER–a) involved in interactions with the Bis-3-chlorobenzyloxychalcone, Bis-2-chlorobenzyloxychalcone compounds, and the reference compound Tamoxifen (TAM)

Compound Name	Residues binding at ligand-protein complex
Bis-3-chlorobenzyloxychalcone compound (PDB ID 6CHZ)	Arg 394; Asp 351; Gly 420; Gly 521; Leu 354; Leu 428; Leu 384; Met 343; Phe 404; Thr 347; Glu 353; Leu 346; His 524; Ala 350; Ile 424; Leu 349; Leu 387; Leu 391; Leu 349; Leu 525; Met 388; Met 421; Trp 383
Pinostrobin compound (PDB ID 6CHZ)	Arg 394; Glu 353; Gly 420; Gly 521; Leu 349; Met 388; Thr 347; Met 421; His 524; Phe 404; Ala 350; Ile 424; Leu 387; Leu 346; Leu 525; Leu 391; Met 343; Trp 383
Chalcone Compound (PDB ID 6CHZ)	Arg 394; Asp 351; Gly 420; Gly 521; Leu 384; Phe 404; Thr 347; His 534; Glu 353; Met 343; Trp 383; Leu 525; Ala 350; Ile 424; Leu 387; Leu 346; Leu 349; Leu 391; Leu 428; Met 388; Met 421
Reference Compound Tamoxifen (PDB ID 6CHZ)	Arg 394; Asp 351; Glu 353; Gly 521; His 524; Ile 424; Leu 354; Leu 384; Leu 391; Met 343; Met 421; Trp 383; Thr 347; Phe 404; Ala 350; Leu 346; Leu 349; Leu 387; Leu 428; Leu 525; Met 388
Compound Bis-2-chlorobenzyloxychalcone (PDB ID 3ERT)	Arg 394; Ile 424; Gly 521; Leu 428; Met 388; Met 528; Phe 404; Thr 347; Asp 351; Glu 353; Leu 525; Met 343; Leu 346; Trp 383; Ala 350; Leu 349; Leu 354; Leu 384; Leu 387; Leu 391; Leu 536; Met 421
Pinostrobin compound (PDB ID 3ERT)	Arg 394; Asp 351; Gly 420; Ile 424; Leu 384; Leu 428; Phe 404; Thr 347; Trp 383; Gly 521; Leu 391; Met 343; Met 421; His 524; Leu 346; Ala 350; Leu 387; Leu 525; Met 388
Chalcone Compound (PDB ID 3ERT)	Arg 394; Asp 351; Glu 420; Gly 419; Gly 521; His 524; Ile 424; Leu 384; Leu 387; Phe 404; Thr 347; Glu 353; Leu 525; Met 343; Leu 346; Trp 383; Ala 350; Leu 349; Leu 354; Leu 391; Leu 536; Met 421; Met 528
Reference Compound Tamoxifen (PDB ID 3ERT)	Arg 394; Asp 351; Glu 491; Gly 420; Gly 521; His 524; Leu 349; Leu 354; Leu 384; Met 343; Phe 404; Trp 383; Thr 347; Ala 350; Leu 346; Leu 387; Leu 391; Ile 424; Leu 428; Leu 525; Met 388; Met 421

Based on the binding energy data (Table 3), it can be concluded that the bond between the two Chalcone derivative compounds, namely Bis-3-chlorobenzyloxychalcone with the PDB ID 6CHZ as the target of the estrogen receptor alpha (ER–a) and Bis-2-chlorobenzyloxychalcone with the PDB ID 3ERT which is also the target of the estrogen receptor alpha (ER–a), is more stable than the parent compound, namely Chalcone (2,6-dihydroxy-4-methoxychalcone), the compound examined is also predicted to have lower cytotoxic activity than the reference compound. Ideally, we would expect hydrogen bonding interactions;

however, we did not observe any of these interactions between the molecules and tamoxifen. Nevertheless, these interactions are represented by the lowest binding energy, which indicates that the drug-protein interaction occurred spontaneously.

Conclusion

Based on the results of this molecular modeling study, it can be concluded that the Bis-3-chlorobenzyloxychalcone and Bis-2-chlorobenzyloxychalcone compounds are the most feasible compounds to be synthesized and continued with in vitro and in vivo tests to determine whether the two compounds exhibit activity against breast cancer cells and test animals. This approach is taken because the two compounds are predicted to have the best activity without causing hepatotoxic effects and other toxic effects.

Acknowledgments
The author would like to express sincere appreciation to the Fakultas Farmasi, Universitas Surabaya, for the invaluable support throughout the research process, including the provision of facilities and resources, which were extremely instrumental in the successful completion of this research project. Finally, the contributions and support greatly enhanced the quality and rigor of this research. The author also extends gratitude to her fellow authors for their assistance in completing this research article. It is hoped that this article will be useful and beneficial to all parties involved.

Author Contributions
All authors had significant contributions in completing this manuscript.

Funding
This research was funded by Program Hibah Penelitian Kemendikbudristek – Penelitian Tesis Magister Tahun Anggaran 2024, grant number 9905.

Conflicts of Interest
The authors declare no conflict of interest.

References

Ali, S., Rasool, M., Chaoudhry, H., N Pushparaj, P., Jha, P., Hafiz, A., Mahfooz, M., Abdus Sami, G., Azhar Kamal, M., Bashir, S., Ali, A., & Sarwar Jamal, M. (2016). Molecular mechanisms and mode of tamoxifen resistance in breast cancer. *Bioinformation*, *12*(3), 135–139. https://doi.org/10.6026/97320630012135

Comşa, Ş., Cîmpean, A. M., & Raica, M. (2015). The Story of MCF-7 Breast Cancer Cell Line: 40 years of Experience in Research. *Anticancer Research*, *35*(6), 3147–3154.

D. Kesuma, Siswandono, & A. Kirtishanti. (2022). Molecular Docking And Biological Activity Of N-(4-Methoxy)-Benzoyl-N'-Phenylthiourea And N-(4- Trifluoro)-Benzoyl-N'-Phenylthiourea As Antibreast Cancer Candidates. *RASAYAN Journal of Chemistry*, *15*(02), 1503–1508. https://doi.org/10.31788/RJC.2022.1526836

Departemen Kesehatan Republik Indonesia. (2017). *Farmakope Herbal Indonesia, Edisi II*. Departemen Kesehatan RI.

Faleye, O. S., Boya, B. R., Lee, J.-H., Choi, I., & Lee, J. (2024). Halogenated Antimicrobial Agents to Combat Drug-Resistant Pathogens. *Pharmacological Reviews*, *76*(1), 90–141. https://doi.org/10.1124/pharmrev.123.000863

Hardjono, S., Siswodihardjo, S., Pramono, P., & Darmanto, W. (2016). Quantitative Structure-Cytotoxic Activity Relationship 1-(Benzoyloxy)urea and Its Derivative. *Current Drug Discovery Technologies*, *13*(2), 101–108. https://doi.org/10.2174/15701638136661605251122327

Hinchliffe, A. (2008). *Molecular Modeling for Beginners* (2nd ed.). John Wiley and Sons Ltd.

Kumar, S., & Pandey, A. K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal*, *2013*(1). https://doi.org/10.1155/2013/162750

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *23*(1–3), 3–25. https://doi.org/10.1016/S0169-409X(96)00423-1

Mass, E. B., de Lima, C. A., D'Oca, M. G. M., Sciani, J. M., Longato, G. B., & Russowsky, D. (2022). Synthesis, Selective Cytotoxic Activity against Human Breast Cancer MCF7 Cell Line and Molecular Docking of Some Chalcone-Dihydropyrimidone Hybrids. *Drugs and Drug Candidates*, *1*(1), 3–21. https://doi.org/10.3390/ddc1010002

Pratama, M. R. F., Praditapuspa, E. N., Kesuma, D., Poerwono, H., Widiandani, T., & Siswodihardjo, S. (2022). Boesenbergia Pandurata as an Anti-Breast Cancer Agent: Molecular Docking and ADMET Study. *Letters in Drug Design & Discovery*, *19*(7), 606–626. https://doi.org/10.2174/1570180819666211220111245

Saputri, K. E., Fakhmi, N., Kusumaningtyas, E., Priyatama, D., & Santoso, B. (2016). Docking Molekular Potensi Anti Diabetes Melitus Tipe 2 Turunan Zerumbon Sebagai Inhibitor Aldosa Reduktase Dengan Autodock-Vina. *Chimica et*

Natura Acta, *4*(1), 16. https://doi.org/10.24198/cna.v4.n1.10443

Schlick, T. (2010). *Molecular Modeling and Simulation: An Interdisciplinary Guide* (Vol. 21). Springer New York. https://doi.org/10.1007/978-1-4419-6351-2

Siswandono. (2016). *Kimia Medisinal 1* (Kedua). Airlangga University Press.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

The Global Cancer Observatory. (2021). *Globocan 2020: Indonesia*. https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf

Yap Li Ching, A., Sook Wah, T., Aspollah Sukari, M., Ee Cheng Lian, G., Rahmani, M., & Khalid, K. (2007). Characterization of Flavonoid Derivatives from Boesenbergia rotunda (L.). *The Malaysian Journal of Analytical Sciences*, *11*(1), 154–159.