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MODOFICATION OF THE STRUCTURE OF CHALCONE DERIVATIVES ISOLATED FROM FINGERROOT RHIZOME (*BOESENBERGIA ROTUNDA*) AS A POTENTIAL ANTICANCER AGENTS FOR BREAST CANCER

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Women worldwide, including in Indonesia, are particularly vulnerable to breast cancer, which is often detected at advanced stages. Existing treatment approaches have weaknesses and low success rates, as the drugs used become less effective and cancer cells can develop resistance. Therefore, we need to innovate to create new anticancer drug candidates from natural sources like the fingerroot rhizome (Boesenbergia rotunda). In this study, in silico testing was conducted to predict cytotoxic activity using AutoDock Vina. The isolation of the rhizome and the synthesis of chalcone derivatives were performed using green synthesis methods through Williamson ether reactions with microwave irradiation. Structure identification was carried out using an IR spectrometer and a ^{1}H-NMR spectrometer. In vitro activity was assessed using MTT assays on MCF-7 cells and normal Vero cells. The results showed that the chalcone compounds, Bis-4-chlorobenzyloxychalcone and Bis-4-bromobenzyloxychalcone, exhibited better bioavailability, toxicity, and activity in silico. In vitro, they demonstrated significant cytotoxic activity compared to tamoxifen against MCF-7 cells and greater selectivity towards MCF-7 cells compared to normal Vero cells. Therefore, Bis-4-chlorobenzyloxychalcone and Bis-4-bromobenzyloxychalcone have the potential to be candidates for anticancer drugs for breast cancer, providing alternative options to reduce the side effects associated with drug use.

Keywords: *Breast Cancer, Chalcone, Fingerroot rhizomes (Boesenbergia rotunda), Green Synthesis, MCF-7*

INTRODUCTION

In Indonesia and in other countries, cancer has become a public health issue. Cancer is a non-communicable disease characterized by the rapid and uncontrolled growth and development of cells and tissues. This growth can disrupt the body's metabolism and spread between cells and tissues[1]. The most frightening cancer for women around the world, including Indonesia, is breast cancer, as it is often found at an advanced stage[2]. About 70% of breast cancer expresses estrogen receptors (ER). Estrogen receptors can be used to determine the sensitivity of breast cancer to anti-estrogen therapy and to assess the sensitivity of preventive chemotherapy in patients at high risk for breast cancer[3]. ER-α is usually associated with increased cell proliferation, making it a potential target for the discovery and development of breast cancer drugs[4]. The MCF-7 breast cancer cell line is

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one of the adherent cell lines that express alpha estrogen receptors[5].

In breast cancer, the primary treatment is surgery, which may be accompanied by chemotherapy or radiotherapy or used alone, followed by hormone therapy[6]. However, each treatment method has its weaknesses, resulting in a low success rate. Meanwhile, medications that have been used for a long time gradually become less effective, and cancer cells tend to become resistant to anticancer drugs[7-9]. Therefore, innovation in the development and discovery of cancer treatments, especially for breast cancer, must continue in order to obtain new, effective, safe, and high-quality cancer drugs. The development and discovery of new cancer treatments can be derived from natural substances that act as chemotherapy agents and enhance the sensitivity of cancer cells, such as the use of the rhizome of fingerroot (*Boesenbergia rotunda*).

Fingerroot rhizomes (*Boesenbergia rotunda*) have the ability to increase the number of lymphocytes and specific antibodies, as well as the ability to kill cancer cells. Flavanon, flavon, and chalcone are the main flavonoids found in the extract of fingerroot rhizomes[10]. In the field of synthesis, chalcone compounds have been widely used to create various types of heterocyclic compounds, such as flavones, isoxazoles, benzodiazepines, pyrazolines and their derivatives, as well as flavonols. This has led to chalcone analogs and their derivatives being extensively used as target molecules in the search for active compounds as drug candidates, one of which is for cancer treatment[11]. Chalcone has the potential to be a chemotherapeutic agent due to its proven strong biological activity; however, compared to other flavonoid compounds, its availability in nature is very limited, necessitating effective synthesis methods[12]. The chalcone compound can be synthesized from pinostrobin obtained from the isolation of the rhizome of the fingerroot rhizomes. The lead compound is structurally modified to obtain a compound with the desired activity. With the modification of the structure, it is hoped that the selectivity and activity of the drug can be increased while reducing toxicity[13].

In this study, an *in silico* test was conducted on chalcone compounds and their derivatives using the estrogen receptor-α with PDB code: 6CHZ. The next stage is the synthesis of chalcone from pinostrobin isolated from the rhizome of the fingerroot rhizomes. The purity of the compound was determined using Thin Layer Chromatography (TLC). If the TLC results showed a single spot, the compound was considered relatively pure. Structure identification was carried out using an IR spectrometer and a ^{1}H-NMR spectrometer. Subsequently, the cytotoxic activity was tested *in vitro* using the MTT microculture assay on MCF-7 cancer cells and normal Vero cells. This study aims to determine the activity of chalcone derivatives as candidates for breast cancer treatment.

MATERIAL AND METHODS

Materials

The materials used in the *in silico* testing phase are active compounds derived from the rhizome of *Boesenbergia rotunda*, specifically chalcones and their derivatives, whose structural images were created using the MarvinSketch application, along with the estrogen receptor-α coded as PDB 6CHZ, and the comparative compound tamoxifen, which was downloaded from the Protein Data Bank site (PDB).

The materials used in the isolation of compounds, synthesis, and structural confirmation stages are key ginger powder (*Boesenbergia rotunda*) which has been determined at the Materia Medica Laboratory in Batu; n-Hexane p.a. (E.Merck, Germany); Ethyl acetate p.a. (E.Merck, Germany); methanol; pinostrobin (compound isolated from fingerroot rhizome powder); Chalcone (2,6-dihydroxy-4-methoxychalcone)(compound synthesized from pinostrobin); 4-chlorobenzyl chloride (TCI, America); 4-bromobenzyl chloride (TCI, America); NaOH p.a. (E-Merck, Germany); Tetrabutylammonium chloride p.a. (E-Merck, Germany); Chloroform (E-Merck, Germany); and anhydrous $MgSO_4$.

The materials used in the *in vitro* test include the compound 2,6-dihydroxy-4-methoxychalcone and its two derivatives substituted with 4-Cl (Bis-4-chlorobenzyloxychalcone) and 4-Br (Bis-4-bromobenzyloxychalcone) from synthesis; Tamoxifen; MCF-7 cell culture and Vero cells; DMEM culture media; MI99 culture media; Fetal Bovine Serum (FBS); Phosphate Buffer Saline (PBS); DMSO; Trypsin; Penicillin-Streptomycin, Fungizone; MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium

bromide) 0.5 mg/mL, SDS 10% in 0.1 N HCl.

Tools and Equipments

The tools used for *in silico* testing include computer hardware with specifications of an Intel(R) Core (TM) i7-9700F CPU @ 3.00GHz, 16384MB RAM, equipped with the Windows 10 Education 64-bit operating system (University of Surabaya), AutoDock Vina and AutoDockTools 1.5.6 programs; PyRx program; MarvinSketch program; Lipinski Rule of Five website; pkCSM website; and BIOVIA Discovery Studio Visualizer.

The tools used for isolation, synthesis, and structural confirmation are glassware, Rotary Evaporator; Waterbath; Corning Hot Plate P351; Fisher-John Electrothermal Mel-Temp; Thin Layer Chromatography UV; Thin Layer Chromatography Plate; Thin Layer Chromatography Chamber; IR Spectrophotometer (Agilent); and ^{1}H-NMR Spectrometer 400 MHz. (Jeol).

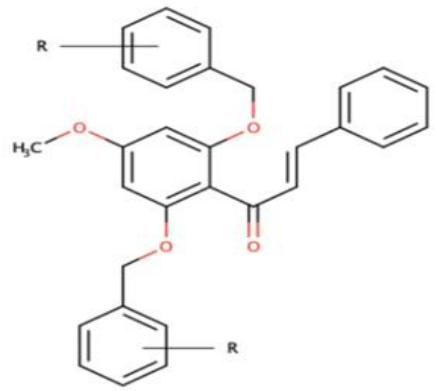
The *in vitro* testing was conducted at the Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University, Special Region of Yogyakarta, Indonesia, 55281. The equipment used for *in vitro* testing includes a CO_2 incubator; LAF (Gelman Sciences); micropipettes of 20, 200, and 1000 µL along with blue and yellow tips; test tubes; vortex; 96-well microplate; conical tube; inverted microscope (Zeiss 451235); hemocytometer; cell counter; and ELISA reader (Bio-Rad).

In Silico

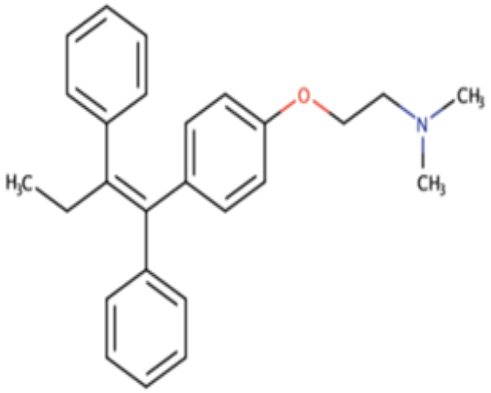
In the *in silico* testing stage, a ligand preparation process was conducted beforehand, where the chemical structure of chalcone and its derivatives was depicted in 2D and converted into 3D using MarvinSketch software and saved in a format (.mol2). (**Fig. 1**) shows the results of the structural modification carried out using the Topliss approach. Modifications were made to chalcone compounds and their derivatives, along with the comparison compound tamoxifen. Next, the receptor preparation process is carried out, where the estrogen receptor-α with PDB code 6CHZ and tamoxifen are obtained from the Protein Data Bank (https://www.rcsb.org/). The receptor preparation process was carried out using AutoDockTools 1.5.6 software.

The next step is to perform docking, but first, a working folder needs to be prepared containing autogrid4.exe and autodock4.exe, as well as the receptor and ligand in .pdbqt format that have been prepared. Molecular docking was performed using AutoDock Vina and Autodock Tools 1.5.6, and then executed using the Command Prompt (CMD). The Pyrx software was also used in this research and can be downloaded from (https://pyrx.sourceforge.-io/). BIOVIA Discovery Studio Visualizer (DSV) software is used to visualizi the docking results of ligand binding with amino acids on the receptor in 2D and 3D. The prediction of toxicity and bioavailability of compounds is carried out through the pkCSM website (https://biosig.lab.uq.edu.au/pkcsm/); website (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp) is used for analyzing the lipophilic properties of compounds based on Lipinski's Rule of Five.

In (**Fig. 1**), the values of the R group can be found in (**Table 1**).



A



B

Fig. 1: Structure of Chalcone Compound (A) and Comparative Compound Tamoxifen (B).

Table 1: The Chemical Structure of The Chalcone Compound.

No.	Position	R	Compound Name
1.	4	Cl	Bis-4-Cholorbenzyloxy chalcone
2.	4	Br	Bis-4-Bromobenzyloxy chalcone

Isolation of Pinostrobin Compound

A total of 2 kg of fingerroot rhizome powder was extracted using 20 L of n-hexane solvent through maceration three times. Evaporate the remaining solvent using a rotary evaporator at low pressure until the volume reaches 1/3 of the initial volume, then use a water bath until coarse crystals are obtained. After that, a recrystallization process was carried out by dissolving the coarse crystals in hot methanol solvent and cooling it in a refrigerator for 24 hours until crystals formed. The crystals that have formed were washed with hot methanol four times, then left to stand at room temperature. After that, filter the crystals and dry them to produce calcium crystals[14]. Characterization of purity testing was carried out using Thin Layer Chromatography (TLC) and melting point determination[15], and identify the chemical structure using an IR Spectrophotometer and a 400 MHz ^{1}H-NMR Spectrophotometer.

Synthesis of Chalcone Compounds

A 12.5 N NaOH solution is made in a glass beaker. The steps for making it can be done by weighing 50 grams of NaOH. (1.250mmol). After that, add 100 mL of distilled water, and stir until homogeneous. Pipette 2 mL of the prepared 12.5 N NaOH solution using a syringe, then transfer it into a round-bottom flask. After that, add 0.2 grams (0.74 mmol) of Chalcone and 0.28 mL (2.22 mmol) of 4-chlorobenzyl chloride and 0.46 mg (2.22 mmol) of 4-bromobenzyl chloride. Add 0.01 grams (0.025 mmol) of Tetrabutylammonium chloride. Heat the mixture in the microwave for 10 minutes at a power level of 200 watts. Purity characterization was carried out using Thin Layer Chromatography (TLC) and melting point determination, while the identification and confirmation of the structure of the isolated and synthesized compounds were performed using IR Spectrophotometry and a 400 MHz ^{1}H-NMR Spectrometer[16-17].

In Vitro

In determining the cytotoxic activity of chalcone derivatives and the comparison compound tamoxifen, an *in vitro* cell growth inhibition test was conducted using MCF-7 cancer cells and normal Vero cells. The first step is to grow all cell cultures in a 96-well plate and incubate them in a CO_2 incubator for 24 hours. After that, each culture well was supplemented with chalcone derivatives and the reference compound tamoxifen at various concentrations and incubated again. Next, the media in the plate is discarded, and the plate is rinsed using 100 µL of PBS, which is then discarded. In the next step, 100 µL of 0.5 mg/mL MTT reagent was added to the microplate, followed by incubation for 4 hours[18]. Then, into each well, 100 µL of 10% SDS in 0.01 N HCl was added to stop the MTT reaction. This is done so that the formazan crystals formed after incubation can dissolve. Next, the microplate is wrapped in paper and incubated for 24 hours at a temperature of 37°C. After that, the absorbance was read with an ELISA reader at a wavelength of 595 nm, and the fraction of live cells was calculated[19]. Probit analysis is used to calculate the IC_{50} values of the reference compound tamoxifen and chalcone derivatives for normal Vero cells and MCF-7 cells.

Statistical Analysis

The percentage of viable cells in the *in vitro* cytotoxicity test was analyzed using probit regression to obtain the IC_{50} value. Probit analysis was conducted using SPSS version 27. (**Table 3**) shows the number of observations and the difference in data in standard deviation (as one example).

Table 2: Binding Energy and Interaction Binding between Chalcone Derivatives and Receptors.

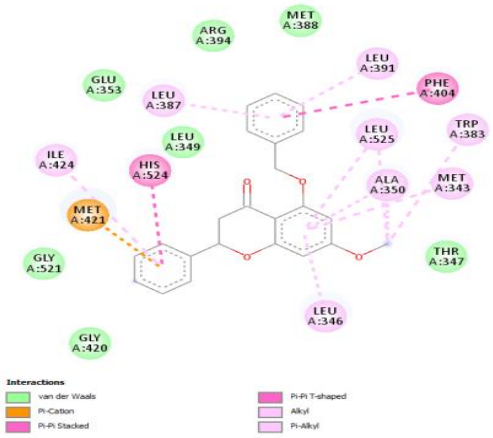
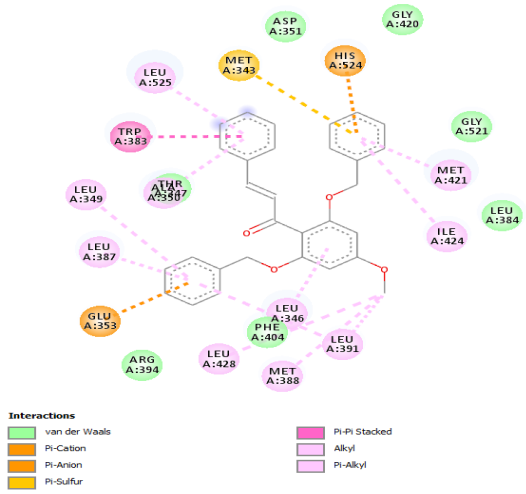
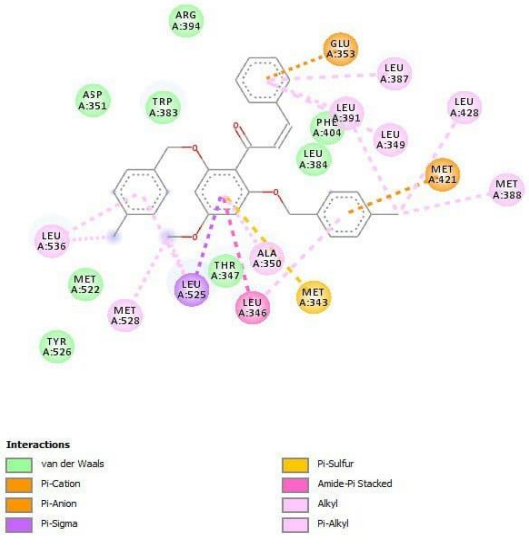
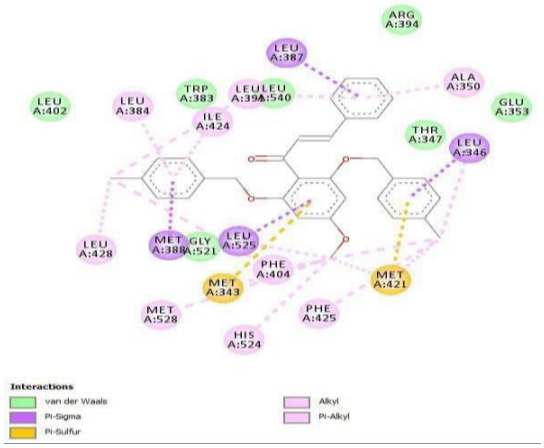
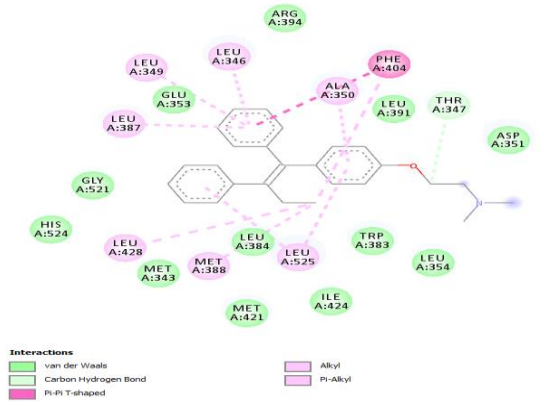
Ligands	Binding Energy (kcal/mol)	Interaction Binding	Amino Acid Residues
Pinostrobin	-10.07		Arg 394; Glu 353; Gly 420; Gly 521; Leu 349; Met 388; Thr 347; Met 421; His 524; Phe 404; Ala 350; Ile 424; Leu 387; Leu 346; Leu 525; Leu 391; Met 343; Trp 383
Chalcone	-		Arg 394; Asp 351; Gly 420; Gly 521; 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
Bis-4-Chlorobenzyl oxychalcone	-		Arg 394; Asp 351; Leu 384; Met 522; Phe 404; Thr 347; Trp 383; Tyr 526; Glu 353; Met 421; Leu 525; Met 343; Leu 346; Ala 350; Leu 349; Leu 387; Leu 391; Leu 428; Leu 536; Met 388; Met 528

Table 2: Continued.

Bis-4-Bromobenzyl oxychalcone	-		Arg 394; Glu 353; Gly 521; Leu 402; Leu 540; Thr 347; Trp 383; Leu 346; Leu 387; Leu 525; Met 388; Met 343; Met 421; Ala 350; His 524; Ile 424; Leu 391; Leu 384; Leu 428; Met 528; Phe 404; Phe 425
Tamoxifen	-10		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

RESULTS AND DISCUSSION

In Silico

The values of binding energy and ligand binding with amino acids can be seen in (**Table 2**). The binding energy value of chalcone and its derivatives can predict the activity of the compounds. The smaller the binding energy value of a compound, the greater its potential activity, and the ligand-receptor bond is stable[20]. The binding energy values for each compound, Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone, are -10.68 kkal/mol and -11.63 kkal/mol, respectively, which are lower than those of Tamoxifen (-10 kkal/mol). Based on the ligand's interaction with amino acids, it can be predicted that the more hydrogen bonds and steric interactions (Van der Waals and hydrophobic) there are between the ligand and the receptor, the more stable the bond between the ligand and the receptor will be[21]. The compound and its derivatives interact with the ER-α receptor, and the interaction occurs through steric binding (Van der Waals and hydrophobic) at the amino acids Arg 394; Asp 351; Gly 420; Gly 521; Leu 384; Phe 404; Thr 347, Leu 384; Met 522, Trp 383; Tyr 526, Glu 353, Leu 402, and Leu 540. Meanwhile, the tamoxifen comparator binds to the ER-α receptor through hydrogen bonds at the amino acid THR 347 and steric interactions (Van der Waals and hydrophobic) at the amino acids 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. Therefore, it can be concluded that chalcone and its derivatives can interact with the ER-α receptor due to their ability to from bonds with the same residues amino acids as tamoxifen, including Arg 394; Asp 351, Gly 521, Leu 384, Trp 383, and Thr 347.

Isolation of Compounds

From 2 Kg of the simplicia powder of the rhizome of fingerroot rhizomes (*Boesenbergia rotunda*), 10.50 grams (0.525%) of pinostrobin crystal isolate was obtained, which is in the

form of white crystals with a yield of 0.525% and a melting point of 99.5 - 100.7°C. After that, the characterization of the compound was carried out to confirm whether the isolated compound was pinostrobin or not. The characterization process was conducted by determining the chemical structure of the compound using IR and ^{1}H-NMR methods. There are five different types of vibrations in pinostrobin's IR spectrum, according to Siswandono, *et al*. (2018)[22]. The first is the –OH phenolic vibration type, which is at 3464 cm^{-3}. The second is the –C=O ketone vibration type, which is at 1699 cm^{-3}. The third and fourth are the –C=C aromatic vibration types, which are at 1661 cm^{-3} and 1598 cm^{-3}, and the fifth and sixth are the –C–O vibration type. Based on the obtained IR spectrum data, specific functional groups characteristic of pinostrobin were identified. The phenolic OH group appeared at 3058 cm^{-1} (C4), while the aliphatic ether bond from methoxy (C2) and the cyclic bond in ring C were observed at wavelengths of 1284–1034 cm^{-1}. The =C–H aromatic bond was detected at 2972–2912 cm^{-1}, and the C=C aromatic bond appeared at 1638–1526 cm^{-1}. Therefore, it can be concluded that the isolated compound is pinostrobin. This conclusion is based on the similarity of the peak patterns and wave numbers of the isolated compound with those of pinostrobin. The IR spectrum can be seen in (**Fig. 2 (A)**).

The ^{1}H-NMR spectrum of the isolated compound revealed a double doublet multiplicity at chemical shifts (δ) of 2.851–3.089 ppm and 5.414 ppm. These ppm values are characteristic of pinostrobin. A chemical shift was also observed at 12.01 ppm, corresponding to the hydrogen bonded to the phenolic OH group. The formation of hydrogen bonds caused the chemical shift to appear in the downfield region (low field). Based on the obtained data, it was concluded that the hydrogen at 2.82 ppm and 3.09 ppm is adjacent to the hydrogen at 5.42 ppm, and vice versa. The IR spectrum can be seen in (**Fig. 3 (A)**). Based on the ^{1}H-NMR analysis, it was determined that the isolated compound is pinostrobin and can be used in the synthesis of chalcone derivatives.

Synthesis of Chalcone Compounds

The synthesis procedure refers to the green synthesis method through a modified Williamson ether reaction using microwave irradiation with a Phase-Transfer Catalyst. (PTC). The synthesis was carried out by reacting pinostrobin with 4-chlorobenzyl chloride and pinostrobin with 4-bromobenzyl chloride.

Bis-4-Chlorobenzyloxychalcone

Melting points 124.8-126.3°C. IR, ν max(cm-1): 2920-2853 (=C-H Aromatic); 1597 (C=C Aromatic); 1207-1103 (C-O Ether). The IR spectrum can be seen in (**Fig. 2 (B)**). ^{1}H-NMR (Chloroform-D, 400 MHz). δ 3.93 (s, 2H); δ 5.04 (s, 5H); δ 6.18 (s, 3H); δ 7.23 (s, 3H); δ 7.34 (d, 1H); δ 7.40 (m, 6H); δ 7.47 (m, 4H). The ^{1}H-NMR spectrum can be seen in (**Fig. 3 (B)**).

Bis-4-Bromobenzyloxychalcone

Melting points 102.8-103.9°C. IR, ν max(cm-1): 2922-2853 (=C-H Aromatics); 1617 (C=C Aromatics); 1203-1101 (C-O Eter). The IR spectrum can be seen in (**Fig. 2 (C)**). ^{1}H-NMR (Chloroform-D, 400 MHz). δ3.85 (s, 3H); δ5.08 (t, 4H); δ6.36 (s, 2H); δ6.89 (d, 1H); δ7.45 (m, 7H); δ 7.62 (m, 6H); δ7.75 (m, 1H). The ^{1}H-NMR spectrum can be seen in **(Fig. 3 (C)).**

Based on the IR spectrum data, it was concluded that the two synthesized compounds are the desired chalcone derivatives, as they exhibit peak patterns and wave numbers similar to those of chalcone. The substitution of 4-chlorobenzyl and 4-bromobenzyl resulted in the replacement of the phenolic OH group with a C–O ether bond. Identification was carried out using ^{1}H-NMR at a frequency of 400 MHz.

Based on the ^{1}H-NMR analysis of each synthesized compound, no chemical shift was observed at 12.02 ppm, indicating the successful formation of Bis-4-chlorobenzyl oxychalcone and Bis-4-bromobenzyl oxychalcone. However, in Bis-4-bromobenzyl oxychalcone, residual pinostrobin or impurities from the solvent used were still detected.

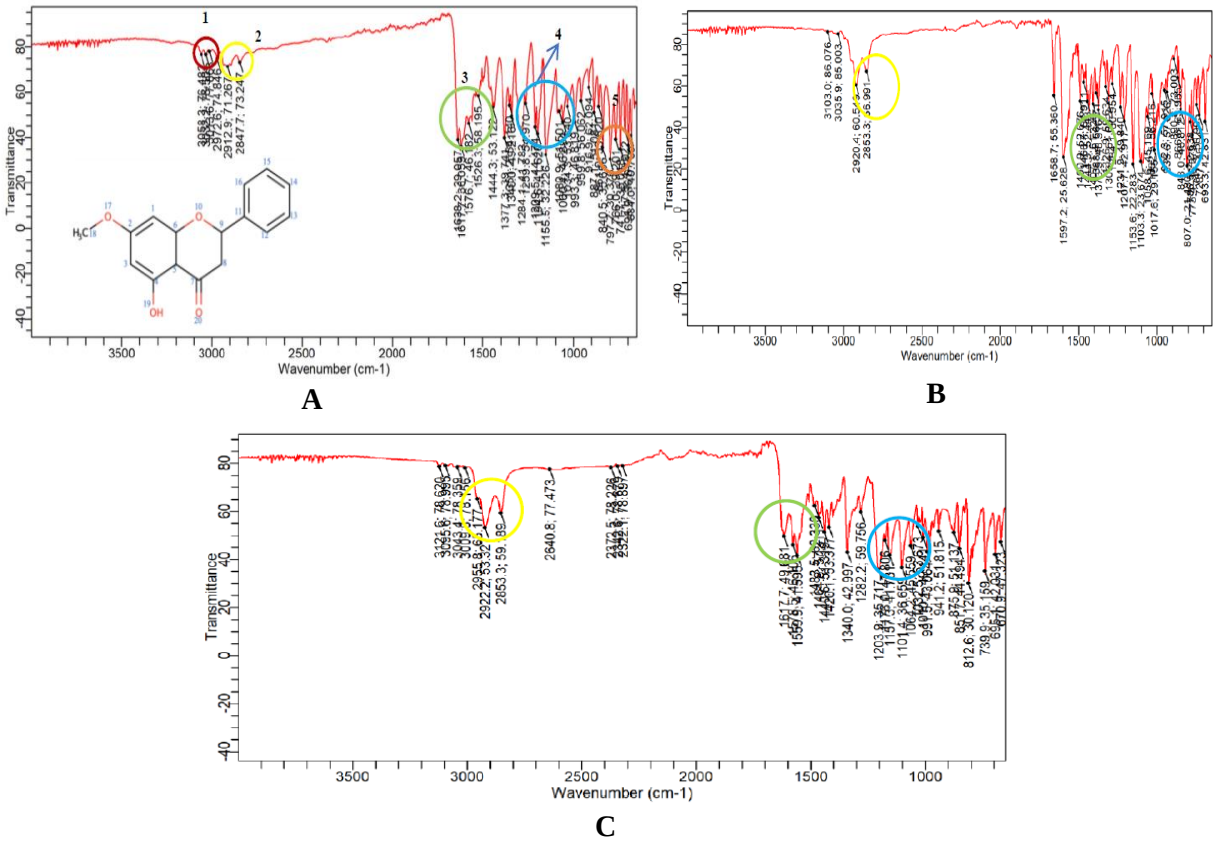


Fig. 2: The IR Spectrum of the Isolated Compound/Pinostrobin (A), the Synthesized Bis-4-Chlorobenzyloxychalcone (B), and Bis-4-Bromobenzyloxychalcone (C).

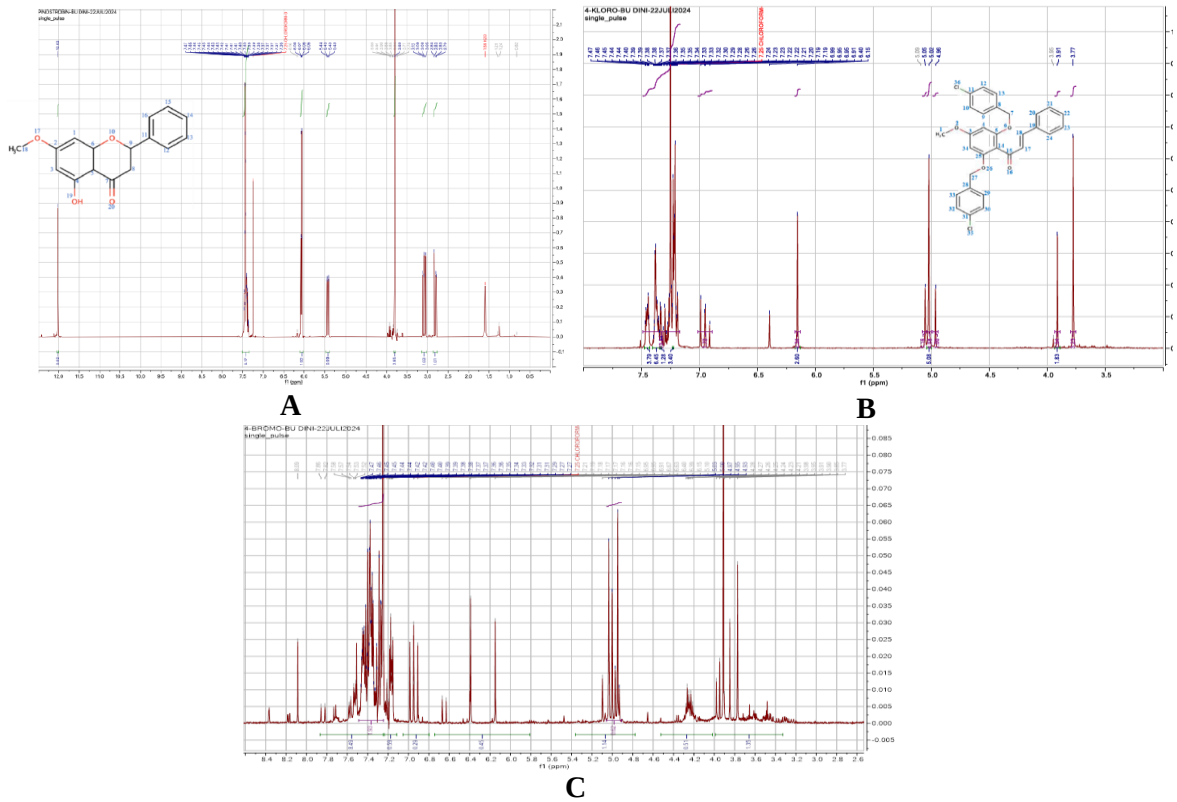


Fig. 3: ^{1}H-NMR Spectrum of the Isolated Compound/Pinostrobin (A), Synthesized Bis-4-Chlorobenzyloxychalcone (B), and Bis-4-Bromobenzyloxychalcone (C).

In Vitro

The summary results of the IC_{50} values for the test compounds and the comparator against MCF-7 cells and normal Vero cells can be seen in (**Table 4**). According to the National Cancer Institute (NCI), a sample with an IC_{50} value of < 30 µg/ml is said to have very active cytotoxic activity. Meanwhile, a sample with an IC_{50} value > 30 µg/ml and < 100 µg/ml is said to have fairly active cytotoxic activity, while a sample with an IC_{50} value > 100 µg/ml is considered inactive or lacking cytotoxic activity[23]. (**Table 4**) shows the IC_{50} values of the test compounds Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone on MCF-7 cells, which are 60.891 µg/mL (0.117 mM) and 53.115 µg/mL (0.087 mM), respectively,while the reference compound tamoxifen has an IC_{50} value of 1.460 µg/mL (0,002 mM). Based on the results obtained, it can be concluded that the compounds Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone exhibit fairly active cytotoxic activity, while the comparative compound tamoxifen shows very active cytotoxic activity against MCF-7 cells. In normal Vero cells, the IC_{50} value of Bis-4-Chlorobenzyloxychalcone was obtained at 1749.631 µg/mL (3.368 mM), the IC_{50} value of Bis-4-Bromobenzyloxychalcone was 697.511 µg/mL (1.146 mM), and the IC_{50} value of the reference compound tamoxifen was 1.472 µg/mL. Based on the results obtained, it can be concluded that the compounds Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone exhibit fairly active cytotoxic activity, while the comparative compound tamoxifen shows very active cytotoxic activity against normal Vero cells.

The low IC_{50} value of chalcone derivatives compared to the reference drug (Tamoxifen) is due to Tamoxifen having a higher affinity for ER-alpha, a more specific mechanism of action, more efficient cellular penetration, and more optimal molecular interactions. Meanwhile, Bis-4-Chlorobenzyl oxychalcone and Bis-4-Bromobenzyl oxychalcone may exhibit biological activity, but their mechanism of action might be less specific to ER-alpha, requiring higher doses to achieve the same effect and necessitating further development. The lower IC_{50} value of chalcone derivatives compared to the reference drug (Tamoxifen) is because Tamoxifen has a higher affinity for ER-alpha, a more specific mechanism of action, more efficient cell penetration, and more optimal molecular interactions[24].

Meanwhile, Bis-4-Chlorobenzylideneacetone and Bis-4-Bromobenzylideneacetone may have biological activity, but their mechanisms of action might be less specific to ER-alpha, requiring higher doses to achieve the same effect and further development.

The Selectivity Index (SI) is a selectivity parameter used to measure the safety of a drug. If calculated using the formula:

$$\textit{Selectivity Index} = \frac{IC_{50}\ \text{normal cell}}{IC_{50}\ \text{cancer cells}} \qquad (1)$$

A selectivity index value greater than 2 indicates that a sample has cytotoxic activity against cancer cells without affecting normal cells[23]. Based on the Selectivity Index data, tamoxifen has an SI value of 1.008, while the compounds Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone have SI values of 28.733 and 13.132, respectively. Based on this, it can be concluded that the reference compound tamoxifen is not selective towards cancer cells and normal cells, whereas the compounds Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone are selective. In other words, the synthesized compounds (Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone) can be used as alternative therapies for breast cancer. However, in terms of their activity, the compounds Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone are only classified as moderately active. Therefore, the compounds Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone can be used as alternative options because they have a good Selectivity Index, which can reduce the side effects caused by the use of the drug.

Table 3: Cytotoxic Test of Bis-4-Chlorobenzyloxychalcone on MCF-7 Cells.

Bis-4-Chlorobenzyloxychalcone									
No.	**Concentration (μg/mL)**	**Absorbance**					**Living Cells (%)**	**Dead Cells (%)**	**IC_{50} (μg/mL)**
		I	**II**	**III**	**Average**	**SD**			
1	6.25	0.398	0.679	0.654	0.577	0.156	77.004	22.996	60.633
2	12.5	0.396	0.468	0.457	0.440	0.039	55.526	44.474	
3	25	0.406	0.359	0.379	0.381	0.024	46.255	53.745	
4	50	0.325	0.349	0.398	0.357	0.037	42.483	57.517	
5	100	0.263	0.391	0.429	0.361	0.087	43.059	56.941	
6	200	0.349	0.621	0.348	0.439	0.157	55.369	44.631	

Table 4: IC_{50} Values of the Test and Comparison Compounds for MCF-7 and Vero Cells and Their Selectivity Index Values.

Compounds	**IC_{50} MCF-7 Cells**		**IC_{50} Vero Cells**		***Selectivity Index* (SI)**
	μg/mL	**mM**	**μg/mL**	**mM**	
Bis-4-chlorobenzyloxychalcone	60.891	0.117	1749.631	3.368	28.733
Bis-4-bromobenzyloxychalcone	53.115	0.087	697.511	1.146	13.132
Tamoxifen	1.460	0.002	1.472	-	1.008

Conclusion

The compound Chalcone and its two derivatives, Bis-4-Chlorobenzyloxychalcone and Bis-4-Bromobenzyloxychalcone, exhibit significantly higher cytotoxic activity against MCF-7 cells *in vitro* compared to Tamoxifen. Additionally, Bis-4-Chlorobenzyl-oxychalcone and Bis-4-Bromobenzyl-oxychalcone demonstrate greater selectivity towards MCF-7 cells than normal Vero cells.

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نشرة العلوم الصيدليـــة
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تعديل البنية الكيميائية لمشتقات الشالكون المفصولة من ريزومة نبات بوسنبيرجيا روتوندا كعوامل مضادة لسرطان الثدي

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تعاني النساء في جميع أنحاء العالم، بما في ذلك في إندونيسيا، من التعرض للإصابة بسرطان الثدي، والذي غالباً ما يتم اكتشافه في مراحل متقدمة. تعانى طرق العلاج الحالية من ضعف فعاليتها وانخفاض معدلات النجاح، إذ تصبح الأدوية المستخدمة أقل فاعلية كما يمكن للخلايا السرطانية أن تطور مقاومة لها. لذلك، من الضروري الابتكار لتطوير مرشحات جديدة لأدوية مضادة للسرطان من مصادر طبيعية مثل ريزومة نبات *بوسنبيرجيا روتوندا*. في هذه الدراسة، تم اجراء اختبار حاسوبى للتنبؤ بالنشاط السُمّي الخلوي باستخدام برنامج AutoDock Vina . كما تم عزل الريزومة وتخليق مشتقات الشالكون باستخدام طرق التخليق الخضراء عبر تفاعلات ويليامسون الإيثرية باستخدام الإشعاع الميكروويفي. وقد تم تحديد البنية الكيميائية باستخدام جهاز طيف الأشعة تحت الحمراء وجهاز طيف الرنين النووي المغناطيسي للبروتون. وتم تقييم النشاط المختبرى باستخدام اختبار MTT على خلايا MCF-7(خلايا سرطان الثدي) وخلايا Vero الطبيعية. أظهرت النتائج أن مركبات الشالكون، ثنائى-٤-كلوروبنزيلوكسى شالكون و ثنائى -٤-بروموبنزيلوكسى شالكون ، قد أظهرت توفرًا حيويًا أفضل وسمّية ونشاطًا أعلى في اختبارات الحاسوب .أما في المختبر، فقد أظهرت نشاطًا سُمّيًا خلويًا ملحوظًا مقارنة بالتاموكسيفين ضد خلايا MCF-7 ، وانتقائية أكبر تجاه خلايا MCF-7 مقارنة بالخلايا Vero الطبيعية. بالتالي، فإن ثنائى-٤-كلوروبنزيلوكسى شالكون و ثنائى -٤-بروموبنزيلوكسى شالكون لديهما القدرة على أن يكونا مرشحين كأدوية مضادة لسرطان الثدي، مما يوفر خيارات بديلة لتقليل الآثار الجانبية المرتبطة باستخدام الأدوية التقليدية.