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THE SYNTHESIS OF CHALCONE COMPOUNDS WITH CL AND BR SUBSTITUENTS AND THEIR POTENTIAL ANTICANCER ACTIVITIES AGAINST MCF-7 BREAST CANCER CELLS

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ABSTRACT

The synthesis of chalcone-based Cl and Br substituents has been successfully synthesized for determining the cytotoxic bioactivities against MCF-7 breast cancer cells. The method of synthesizing the chalcone compounds was carried out by Clasien-Schmidt condensation via aldol condensation aldol. The determination of chalcone chemical structures was conducted via elucidated structure. Characterizations of pure compounds were conducted via ^{1}H-NMR, FTIR, Mass spectrophotometer, and Spectrophotometry UV-VIS. The cytotoxicity behaviours were investigated via the addition of Prestoblue^{TM} reagents, while the IC_{50} score was also included as the parameter of investigation. Our findings show that three chalcone compounds yielded for 60.00%, 60.51%, and 74.38% for (E)-3-(4-chlorophenyl)-1-o-tolilprop-2-en-1-on (**1**), (E)-1,3-bis(3-bromophenyl) prop-2-en-1-on (**2**), and (E)-3-(4-bromophenyl)-1-(3-chlorophenyl)prop-2-en-1-on (**3**) respectively. The synthesis results were in accordance with the target compounds, in which the characteristics of the chalcone compounds of C-Cl and C-Br were confirmed via FTIR spectra, ^{1}H-NMR shows 8 signals which equal to 10 hydrogen atoms, indicated. The cytotoxic activities based on IC_{50} for chalcones **1, 2,** and **3** were 3.66 µg/mL, 51.05 µg/mL, and 21.62 µg/mL respectively with the potential for anti-breast cancer via MCF-7 cells via the morphological changes.

Keywords: Chalcone, MCF-7, Presto Blue, Cytotoxic.

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INTRODUCTION

Cancer is cell abnormalities that occurred due to a genetic mutation within the DNA. As it is uncontrollable in which the abnormal growth cells spread (metastases) into other parts of the body. The main feature of cancer cells is characterized by the losing growth control as well as the development of the cancerous cells.[1] The cancer cases in Indonesia (136.2/per 100,000 people) have been reported in the eight ranks in south-east Asian. Whilst, in Asia, the rank is 23 which shows high prevalence. In Indonesia, males with lung cancer accounted for 19.4 per 100,000 people with the death average rate being 10.9 per 100,000 people. The next high prevalence is liver cancer with 12.4 per 100,000 people with a death average rate of 7.6 per 100,000 people. Whilst, for the female, breast cancer is the top reason of mortalities (17 per 100,000 people) with cases of 42.1 per 100,000 people. The second reason for mortalities is cervical cancers (23.4 per 100,000 people) with death average rates of 13.9 per 100,000 people.[2]Thus, breast cancer is a serious threat to females. The chalcone is a subclass of flavonoid material that is commonly found in vegetables, fruits, teas, and any herbal plants.[3] The chalcone is a simply natural chemical structure, and its spread in Indonesia is wide in form of vegetables, teas, and tropical plants.

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The chalcone has a 1.3-diaryl-2-propen-1-on common structure with two aromatic rings connected to three carbon atoms within the α, β-non saturated bonds.[4,22]The chalcone is one of the most interesting biological compounds that have attracted scientists recently. In chalcone-containing plants, a little amount and difficulties were found during the isolation techniques, as well as various and limited chalcone that have been isolated. Several reasons occurred behind these difficulties, such as (1) synthesizing a new compound, (2) preparing the stock, (3) scientific methods, (4) limited structures, and (5) elucidated structures. One of the attempts that have been tried to overcome cancer illness is by seeking the effective and specific uses of anticancer drugs via the synthesis of chalcone compounds. Chalcone itself has attracted scientists in recent decades due to its ability as cytotoxic for cancer line T47D[5], antimicrobial[6], antibacterial[9], toxicity[8], antioxidant[9], antiinflammation[10], anticancer[11,23],and antitumor.[3] Previous studies have synthesized chalcone compounds to assess their bio-activities such as (E)-1-(5-Benzoyl-6-methylindolizin-7-yl-3-(3-fluorophenyl)prop-2-en-1-one (a) and (E)-1-(5-Benzoyl-6-methylindolizin-7-yl-3-(3-chlorophenyl)prop-2-en-1-one. These two compounds were reported to have anticancer activities.[12]Another chalcone compounds that have been synthesized with antitumor characteristics were 6-Acetyl-2-mercapto-5-methyl-3-phenylthieno[2,3-d]pyrimidin-4(3H)-one, 6-Acetyl-3-(4-chlorophenyl)-2-mercapto-5-methylthieno[2,3-d]pyrimidin-4(3H)- one, 6-Acetyl-3-benzyl-2-mercapto-5-methylthieno[2,3-d]pyrimidin-4(3H)-one, 2-Mercapto-5-methyl-3-phenyl-6-(3-(p-tolyl)acryloyl)thieno[2,3-d]pyrimidin- 4(3H)-one, 2-Mercapto-5-methyl-3-phenyl-6-(3-(3,4,5-trimethoxyphenyl) acryloyl) thieno [2,3-d]pyrimidin-4(3H)-one. Jawad *et al.* have also synthesized chalcone-based material (2'hydroxy-2,3,5'-trimethoxychalcone) which has the potential as an anti-breast-cancer, and the (E)-1-(3,4-Dihiydroxyphenyl)-3-(2,5-dimethoxyphenyl) prop-2-en-1-one has been synthesized as antioxidants.[13]Elmonaem et al., 2018 have synthesized -(4-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenoxy)- 1-phenyl-1H-tetrazole with IC_{50} of 45,3 µg/ml and its ability as anticancer products.[14]Similar compounds via the BSLT method have produced 37,05 µg/ml of IC_{50} which shows potential ability as an anticancer.[15]Several derivatives of chalcone particularly in the use of Cl and Br substituents have been synthesized, and some of these derivatives have shown interesting bioactivity results which potentially can be used in clinical tests against diseases, including Br for its anticancer characteristics.[24]Thus, this present work attempts to synthesize three chalcone compounds with their cytotoxic activities as new anticancer drugs and to show their potential as anti-breast-cancer drugs.

EXPERIMENTAL

Materials

The chemical compounds that were used in this study were 4-methylacetophenonE, 4-chloro benzaldehyde, 3-Bromo acetophenone, 3-Bromo benzaldehyde, 3-chloro acetophenone, 4-Bromo acetophenone, sodium hydroxide, Kalium hydroxide, chloride acid, n-hexane, ethyl acetate, methanol, absolute ethanol, dimethyl sulfoxide, nutrient agar, nutrient broth, alcohol 70%, and doxorubine (control positive), and all of these compounds were in analytical grade (supplied by Merck). Meanwhile, acetone, methylene chloride, and chloroform were used in form of technical grade.The cancer testing employed the cell line cancer mcf-7 that was supplied by Medium Rosewell Park Memorial Institute (RPMI) 1640 (GIBCO BRL). The phosphate-buffered saline (PBS), prestoBlue™ cell viability reagent, fetal bovine serum (FBS), trypsin-EDTA, teypan Blue, Cisplatin (for control positive), filter paper with size 0.2µm, and distilled water were in analytical grade.

Synthesis Chalcone via Clasien-Schmidt Condensation

The synthesis of (E)-3-(4-chlorophenyl)-1-o-tolilprop-2-en-1-on (1)

As much as 5 mmol of 2-methylacetophenone was prepared as the aromatic ketone, and the 5 mmol of -4-chlorobenzaldehyde as aldehyde aromatic was prepared to mix into an Erlenmeyer glass. Then, this mixture was distilled with 15 ml of ethanol and mixed in constant stirring. To obtain a homogenized mixture, then 10 ml of sodium hydroxide (50%) was added dropwise. Then, this mixture solution was irradiated in a microwave for 8 minutes with 700 Watt at 80°C, with an interval of 10 seconds to avoid evaporation of this solution.The reaction stage was then placed on thin-layer chromatography with the support of n-hexane/ethyl acetate as the movement phase. Then after the reaction is completed, it is indicated by the aldehyde, ketone, and chalcone with the different movements. Then, this resulting

mixture was allowed to stand for 20 hours to maximize the obtaining precipitation. Due to acidity, as many as 15 ml of the low- temperature of distilled water was added, followed by 10% HCl to adjust the pH to 7. Afterward, the precipitation appears to be formed, and then this solution was filtered by the Buchner funnel. The precipitation then was washed with cold hexane, was dried in a vacuum chamber. The resulting product was tested in terms of purification via thin-layer chromatography, melting point, and HPLC analysis, then the chemical structures were confirmed by spectrophotometer UV, IR, MS, and ^{1}H-NMR spectroscopy.[16]

The synthesis of (E)-1,3-bis(3-bromophenyl)prop-2-en-1-on (2)

The synthesis of this compound was carried out similarly to the previous steps with slight modification. Briefly, the 5 mmol of 3-bromoacetophenon and 3-bromobenzaldehyde respectively as ketone and aldehyde aromatic were mixed together in an Erlenmeyer glass. This mixture then was dissolved in 15 ml of ethanol with constant stirring to obtain a homogenous solution, in which subsequently the 10 ml of KOH (50%) was dropped wisely. Afterward, this mixture was irradiated for six minutes at 80°C for 700 W with an interval of 10 s to avoid solvent evaporation.Then, this solution was transferred into thin-layer chromatography (n-hexane/ ethyl acetate as the movement phase) which was completed by the movements of aldehyde, ketone, and chalcone. This solution was then allowed to stand for 20 hours to maximize the precipitation. Afterward, 15 ml of cold distilled water was poured into the mixture, and 10% HCl was poured to obtain neutral pH. The forming precipitation was then removed by using the Buchner funnel, which later was washed with cold n-hexane. The precipitation was dried in a vacuum chamber, and this solid sample was tested to determine its purity, melting point, and HPLC analysis as well as its structure via spectrophotometer UV, IR, MS, and spectroscopy ^{1}H-NMR.[16]

The synthesis of (E)-3-(4-bromophenyl)-1-(3-chlorophenyl)prop-2-en-1-on (3)

A similar procedure to the synthesis of (E)-3(4-chlorophenyl)-1-o-tolilprop-en-1-on (1) was employed. In brief, as many as 5 mmol 3-chloroacetofenon as aromatic ketones and 5 mmol 4-bromobenzaldehyde as aldehyde aromatic were mixed in Erlenmeyer glass, and this mixture was dissolved in 15 ml of ethanol at constant stirring. After being stirred constantly, this mixture solution was homogenous, and 10 ml of KOH (50%) was dropped wisely into the solution. The resulting solution was then irradiated for four minutes at 700W at 80°C in a microwave, with an interval of 10s to avoid evaporation.The reaction was then placed into a thin layer of chromatography to be observed within n-hexane/ ethyl acetate. Similarly, to the previous procedure, this mixture of the solution was allowed to stand for 20 hours to maximize the precipitation. Then, a-15-mL of cold distilled water was poured into the solution, and to obtain pH 7, 10% HCl was added. The appearing precipitation separated via the Buchner funnel, and then these amounts of precipitation were washed with cold n-hexane which would be dried in a vacuum chamber. The resulting solid samples were characterized to determine their purity, melting points, and HPLC analysis as well as the structure via spectrophotometer UV, IR, MS, and spectroscopy ^{1}H-NMR.[16]

Cytotoxic Test

The cytotoxic test that was applied to determine the anticancer behaviours was employed via cell lines MCF-7 and Vero cells. The culture cell was placed in 96 well-plates, and the incubation condition was designed at 37°C with the airflow of 5% $CO_2$to reach 70% of the cell's growth. After reaching this percentage, the culture cell was incubated for the next 48 hours at the same temperature and CO_2 flow. Then, the working reagent of blue presto was added to the culture. The absorbance measurement was employed via Multimode Reader.[17]

RESULTS AND DISCUSSION

Synthesis of Chalcone Compounds via Condensation Method of Clasien-Schmidt

Synthesis of (E)-3-(4-chlorophenyl)-1-o-tolilprop-2-en-1-on (1)

The results of the synthesis process were some solid crystals with yellow colour. Their mass was 0.786 g (60.00%) with a melting point of 72-73°C. The thin-layer chromatography (TLC) test showed that the value of Rf was 0.72, in which the test employed eluent of n-hexane: ethyl acetate (9:1). The HPLC measurement showed a single peak t_R = 12.5 minutes with wavelengths of 202 and 307 nm. Spectrophotometer UV-Vis findings showed maximum absorption at 308 nm with 0.850 absorbances.

The FTIR spectra (in the KBr plate) confirmed the bonding vibration at wavenumbers of 815 cm^{-1} (C-Cl), 1506 cm^{-1} (C=C of benzene ring), 1595 cm^{-1} (C=O of ketones), 3055 cm^{-1} (C-H of benzene rings), and 3448 cm^{-1} (overtone of C=O). Whilst, the ^{1}H-NMR tests ($CDCl_3$, 500 MHz) depicted 6 signals which equal to 13 hydrogen atoms. These signals were at chemical shifting of δ_H 7,469 ppm (d: 8; 1H); 7,43 ppm (d: 16; 1H), 7,35-7,47 ppm (m, 4H), 7,21 ppm (d: 7,5; 1H); 7,10 ppm (d: 16; 1H); 2,38 ppm (s; 3H). The mass spectrophotometer results (HR-MS) showed m/z of 256.0661 with a chemical formula of $C_{16}H_{13}OCl$.

Synthesis of (E)-1,3-bis(3bromophenyl)prop-2-en-1-on (2)

The successful synthesis of the (E)-1,3-bis (3 bromphenyl) prop -2-en-1-on (2) were yellow solid crystals with 1.1010 g (60.51%) of mass. The melting point of this chemicals was 70-80°C. The TLC measurements showed RF value which was 0.88 with eluent system of n-hexane: ethyl acetate (9:1). A single peak was observed via the HPLC measurements at t_R15 minutes with 208 nm and 308 nm of wavelengths. The UV-Vis spectra depicted maximum absorption at 0.755 with 322 nm of wavelength, whereas the FTIR spectra (in KBr plate) confirmed the bonding vibration at 785 cm^{-1} (C-Br), 1475 cm^{-1} (C=C from benzene rings), 1604 cm^{-1} (C=O from ketones), 3061 cm^{-1} (C-H from benzene rings). The ^{1}H-NMR test ($CDCL_3$, 500 MHz) characterized 9 signals which equalled to 10 hydrogen atoms. The signals were at chemical shifting of δ_H 8,14 ppm (s; 1H); 7,94 ppm (d: 7,5; 1H); 7,802 ppm (s: 1H); 7,55 ppm (dd: 1,5; 1,5; 1H); 7,45 ppm (d; 15,5; 1H); 7,74 ppm (d: 16; 1H); 7,73 ppm (dt: 2; 1; 1H); 7,399 ppm (t: 8; 1H); 7,309 ppm (t: 8; 1H). The mass spectrophotometer (HR-MS) identified the chemical formula of $C_{15}H_{10}OBr_2$ with m/z: 363.9096.

Synthesis of (E)-3-(4-bromophenyl)-1-(3-chlorophenyl) prop-2-en-1-on (3)

The results of the synthesis process were some solid crystals with yellow colour. Their mass was 1.1899 g (74/38%) with a melting point of 72-73°C. The thin-layer chromatography (TLC) test showed that the value of Rf was 0.88, in which the test employed eluent of n-hexane: ethyl acetate (9:1). The HPLC measurement showed a single peak t_R = 15 minutes with wavelengths of 206 and 308 nm. Spectrophotometer UV-Vis findings showed maximum absorption at 323 nm with 0.804 absorbances. The FTIR spectra (in the KBr plate) confirmed the bonding vibration at wavenumbers of 823 cm^{-1} (C-Cl), 794 cm^{-1} (C-Br), 1489 cm^{-1} (C=C of benzene ring), 1604 cm^{-1} (C=O of ketones), 3062 cm^{-1} (C-H of benzene rings), and 3448 cm^{-1} (overtone of C=O). Whilst, the ^{1}H-NMR tests ($CDCl_3$, 500 MHz) depicted 8 signals which are equal to 10 hydrogen atoms. These signals were at chemical shifting of δ_H 8,13 ppm (s: 1H); 7,93 ppm (d: 8; 1H); 7,77 ppm (d: 15,5; 1H); 7,71 ppm (dd: 8; 1; 1H); 7,59 ppm (d: 8,5; 1H); 7,44 ppm (d: 15,5; 1H); 7,40 ppm (d: 3; 1H); 7,42 ppm (t: 3; 1H). The mass spectrophotometer results (HR-MS) showed an m/z of 319.9602 with a chemical formula of $C_{15}H_{10}OClBr$.

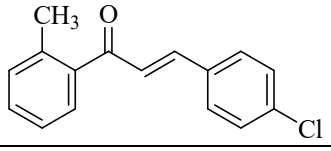
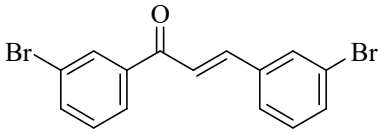
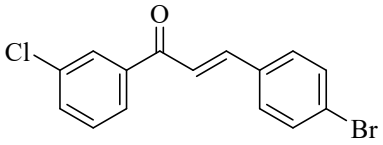
RESULTS AND DISCUSSION

Cytotoxic Test Results to MCF-7 Cancer Cells with Presto Blue™ Reagents

The Reagent Presto Blue™ is a resazurin based-solution. This solution has the characteristic to utilize the living cells reduction as a basis to calculate the cell proliferation quantitatively. When some resazurin is added to the cells, the multimode reader would measure the absorbance behavior of the cells. Thus, living and healthy cells would maintain the pre-reduction environments within their cytosols. After the resazurin enters the living cells, it would be reduced into resorufin with red color-emission with high fluorescent behaviors. The cell's condition could be observed via the change in both fluorescence and absorbance. The active cells that metabolize continuously would change the Presto Blue™ reagent. Whilst, the inactive cells would not be able to change the reagent which has no signals change. The higher intensities of purple emission indicate a higher amount of living cells. The following Table-1 shows the result of the IC_{50} measurement. The breast cancer cells that were used as the sample tests were the MCF-7. These cells originated from the breast tissues of a 69-years-old female with a blood type of O (Rh Positive). The MCF-7 cells were grown in culture media, which have been as cytotoxic samples as anti-cancer agents in vitro. The MCF-7 cells have certain characteristics, such as resistance to chemotherapy agents, ability in expressing alfa estrogen receptor (ER+) which is resistant to doxorubicin, overexpression of Bcl2, and not-expressing caspase-3.[18] The selection of culture media in cytotoxic tests has been designed

intentionally to specific cells for testing. DMSO was used as a control negative as well as a solvent which was dissolved effectively. This could have occurred as its polarity as well as non-polarity, and it may improve sample dissolution. Whilst, whereas cisplatin a control positive which is a pure anticancer agent and comparative substance.[19]The cytotoxic tests of these three compounds could be found in Table-1.

Table-1: IC_{50}Measurement Results

Compounds	Chemical Structure	Regression Linear	The Score IC_{50} (ppm)*
1		y = -0,0102x + 0,2974	3.66
2		y = -0,0064x + 0,5909	51.05
3		y = -0,0221x + 0,7519	21.62

The results of antiproliferation via regression linear equation which defines the concentration of the solution and the percentage of living cells. In accordance with National Cancer Institute (2015), a chemical that is defined to have cytotoxic activities is those that have IC_{50} below 20µg/mL (≤ 20 µg/mL). Based on the IC_{50} score, compounds1 and 3 had cytotoxic activities against MCF-7 cells as their scores were below 20 µg/mL. The compound that had the strongest IC_{50} was compound 1 with an IC_{50} score of 3.66 µg/mL. It has been suggested that the IC_{50} with a score of ≤ 100 µg/mL could be considered to have anticancer activities. Subsequently, this type of compound has the potential to inhibit cell proliferation, which acts as a chemo-prevention agent. In this study, the MCF-7 test results showed resistance characteristics to a few chemotherapy agents.Breast cancer appears to have complex morphological and molecular characteristics.[25]The distinguishing morphological features of MCF-7 breast cancer cells could be observed via concentration parameters. Thus, a high concentration could be observed indicating the death of cells. The observable living cells may be depicted as long shapes, located on the basis of the plate; in contrast; the death cells shape small spherical forms on the surface of the media. The activities tests against the MCF-7 breast cancer cells display a decrease in living cells by the time the increasing concentration.[20]The chalcone is a chemical compound that has flavonoid and tannin contents. The flavonoid features may increase the p53 that enables apoptosis. Unlike flavonoids, tannin may increase the p27 which reduces the cell's cycle in regardto changes in permeability within MCF-7 cells. These changes could be depicted via changes in morphological cells after being added to the compounds in the MCF-7 cells. Moreover, the changes could be also seen as the trypan blue color to the cells which are impermeable to cell membranes. On the treatments, a linear morphological change in the MCF-7 cells may be caused by the increase in concentration tests. The MCF-7 on the control group appears to have an oval with clean cytosol and attaches to the tissue culture dish (TCD). After being treated, some of the cells appear to be rounded cells unattached to the TCD. The cells seem to be cloudy and compact with condensation and shrinkage on the nucleus and granulation within the cytosol. Thus, the treatment of single and combination compounds may also decrease the viability of MCF-7 cells linearly to concentration.

Compound 1 had an IC_{50} score of 3.66 µg/mL which has the lowest score among all compounds. The higher the score of IC_{50}, then the compounds are more toxic and have the potential as anticancer agents with further measurements.

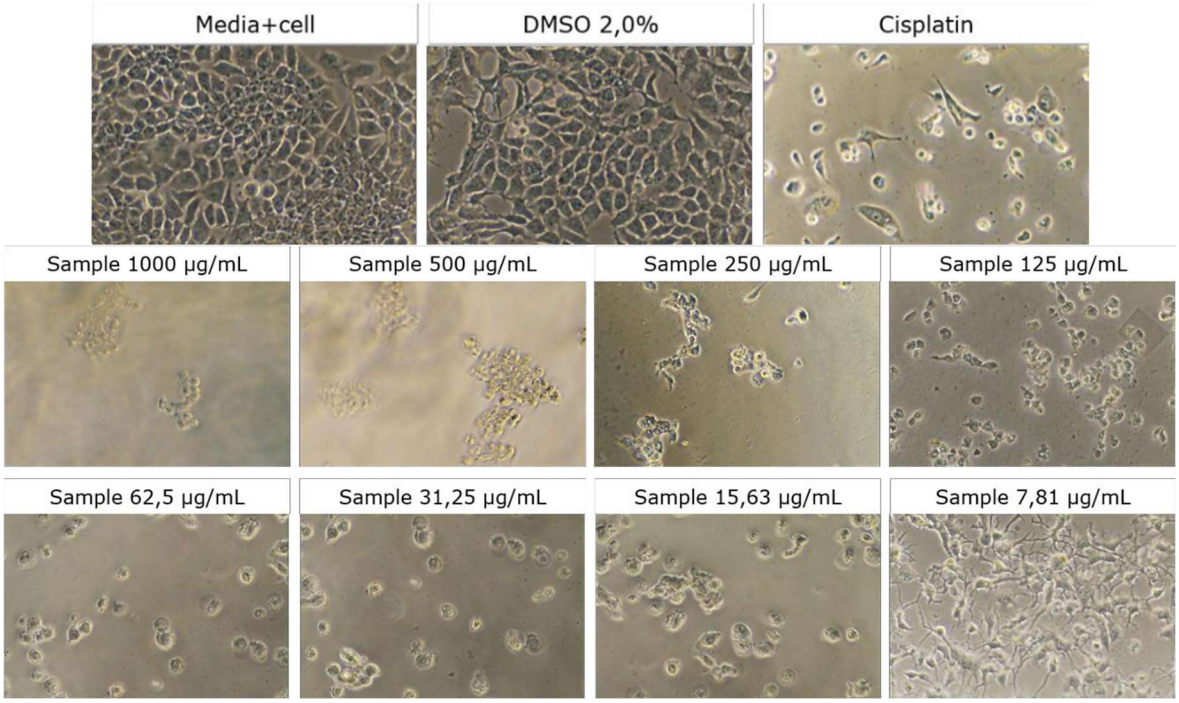


Fig.-1: The Morphological Features of MCF-7 Cells on Compound 1

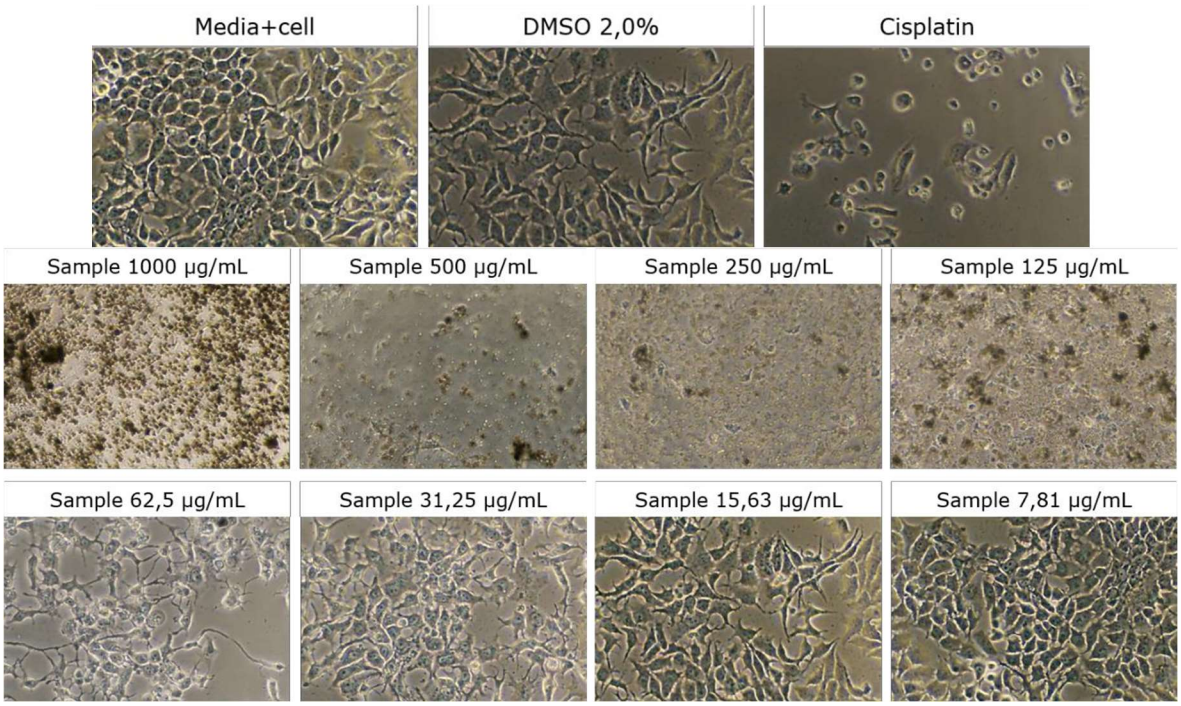


Fig.-2: The Morphological Features of MCF-7 Cells on Compound **2**

The control positive that has been selected in this study was cisplatin (CIS platinum or cis diamminedichloroplatinum(II)). This drug is a common chemotherapy cancer drug based on platina metals. Cisplatin works as an anticancer by attaching itself to the DNA of cancerous cells and preventing cancer growth. The cytotoxic test is the early attempt in detecting compounds that work as antineoplastic drugs within the cytotoxic mechanism. As the parameter measurement is IC_{50} shows the concentration value that allows proliferation inhibition to 50% or potential toxicity.[26] The biological activities of

chalcone in this study were influenced by the presence of α, β-non saturated carbonyl groups and attached substituents on the aromatic rings within the chalcones. Compound 1 has the highest toxicity behavior among all samples which this behavior is due to the methyl (-CH_3) substituent as electron supporter groups. The presence of -CH_3 may increase the electron densities within the aromatic rings so that the compound would be more electronegativity. Subsequently, the carbon atom within the carbonyl would be more electropositive. On the other hand, the halogen substituent that is attached to the aromatic rings generates a negative induction effect (I^-) due to the electronegativity of the halogen atom. Moreover, the same condition allows the compound to generate positive mesomeric effects (M^+) which have been contributed by the presence of free electron pairs on halogen substituents. Therefore, the negative induction effects allow an increase in the chalcone reactivity that may interact with the cellular receptor.[21]

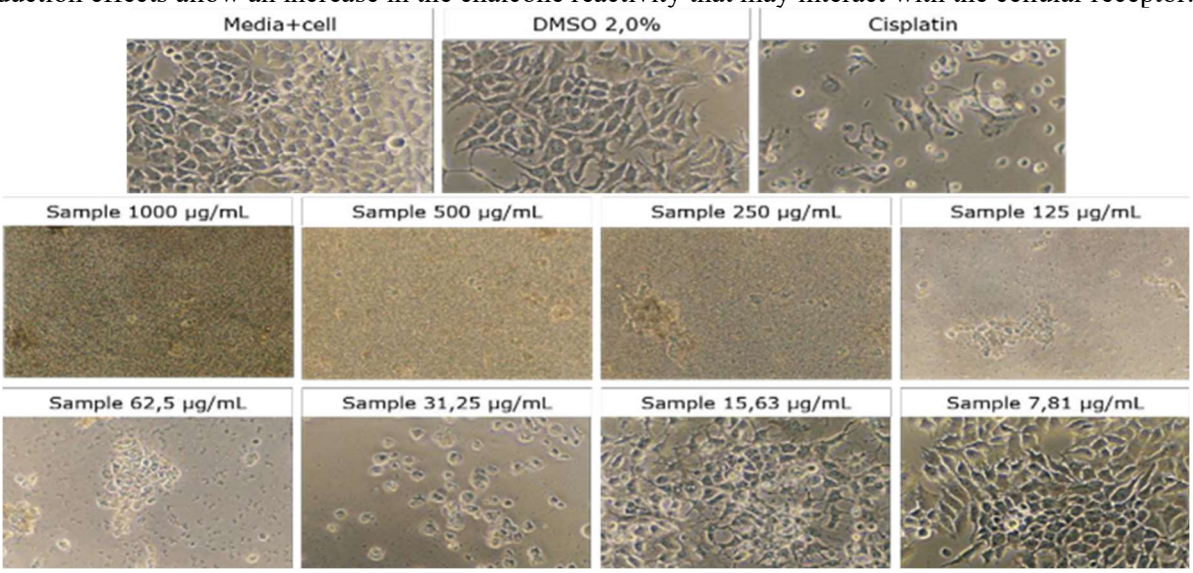


Fig.-3: The Morphological Features of MCF-7 Cells on Compound **3**

CONCLUSION

Based on the study findings, the three synthesized chalcone compounds substituted with Cl and Br 1-3 (E)-3-(4-chlorophenyl)-1-o-tolilprop-2-en-1-on (1), (E)-1,3-bis(3bromophenyl)prop-2-en-1-on (2) dan (E)-3-(4-bromophenyl)-1-(3-chlorophenyl)prop-2-en-1-on (3)) has been successfully synthesized with characteristics, and they have been confirmed via FT-IR, Mass Spectroscopy and ^{1}H-NMR. The synthesis chalcone 1-3 based on halogen substituted has yielded 60.00%, 60.51%, and 74.38% respectively. The bioactivity cytotoxic of the compound 1-3 against the breast cancer cells of MCF-7 accounted for 3.66 µg/mL, 51.05 µg/mL, and 21.62 µg/mL respectively.

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