Synthesis and Cytotoxic Test of Halogen-Substituted Chalcone Against MCF-7 Breast Cancer Cells

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Abstract: *A series of chalcones was synthesized to be (E)-1-(4-chlorophenyl)-3-p-tolyprop-2-en-1-on (1), (E)-1-(4-chlorophenyl)-3-(4-tolyprophenyl) prop-2-en-1-on (2), (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on (3), and (E)-1-(3-bromophenyl)-3-(4-isopropyl phenyl) prop-2-en-1-on (4) using irradiate microwave method with reaction time from 3, 6, and 8 min at 800 °C and 700 W. The compounds were characterized using TLC, UV-Vis, FTIR, ^{1}H-NMR, and evaluated MCF-7 cancer cell cytotoxic test with Presto BlueTM. All compounds produced were in the form of yellow crystals. The results showed that compounds 2, 3, and 4 were potentially a Como prevention agent and inhibit cell proliferation with IC_{50} values of 37.24, 422.22, and 22.41 ppm, respectively. While compound 1 had IC_{50} 1,484.75 and no cytotoxic effect. Further tests should be carried out for compounds 2, 3, and 4 against normal cells to measure the compound's safety for normal cells.*

Keywords: *breast cancer; chalcones; MCF-7 cancer cell*

■ INTRODUCTION

Chalcones are aromatic compounds found from ferns to higher plants and have been used in traditional herbal medicine for centuries. They have an unsaturated side chain and are often cytotoxic in vitro. They also have a highly pleiotropic molecule that can interact with huge molecular targets and have a broad biological spectrum and pharmacological activities [1-2]. As an important class of natural compounds belonging to the flavonoid family, chalcone and its derivative gain significant attention from researchers to be antifungal, anti-inflammatory, antituberculosis, antihyperglycemic, antimalarial, antileishmanial, and anticancer. Its structure consists of two aromatic rings (A-ring and B-ring) linked by a three-carbon alpha, beta-unsaturated carbonyl system [3-4].

The general structure of chalcones consists of coumarin chalcones (III) acting as anticancer agents in the treatment and prevention of cervical, oral squamous, lung, prostate carcinoma, and brain tumors without harming normal cells [5]. Furthermore, chalcones have anticancer activity due to having a similar mode of action to the structurally related natural combretastatin A-4. The methoxy substituent in A ring is used in pharmacology for anticancer potency by inhibiting tubulin polymerization. Anticancer activity is significantly changed by introducing different substituents on aromatic rings of chalcone [3].

Cancer is an uncontrolled cell growth that occurs in invasion into surrounding tissues and spreads (metastatic) to other parts of the body [6]. The number of cancers in Indonesia is still high, ranking 8 in

Southeast Asia, while in Asia is at 23. Global Observatory data 2018 from the World Health Organization (WHO) shows that the most common cancer case in Indonesia is breast cancer, 16.7% of the total 348,809 cancer cases [7]. Therefore, there is a continuous need to develop anticancer drugs that provide maximum therapeutic effect without causing resistance. In plants, the content of chalcone is low, and it is difficult to isolate in large quantities. Besides that, the variation of the structure of chalcone is limited.

Wang et al. [3] synthesized a derivative chalcone containing diaryl ether moiety. They found that a compound with 4-methoxy substitution on the right aromatic ring has a high activity on MCF-7, HepG2, and HCT1 16 cancer lines, with IC_{50} values of 3.44 ± 0.19, 4.64 ± 0.23, and 6.31 ± 0.27 μM, respectively. Luo et al. [8] synthesized a series of novel ligustrazine-chalcone hydrides and evaluated in vitro and in vivo antitumor activities. It showed that these compounds exhibited significant in vitro cytotoxicity against MDA-MB-231, MCF-7, A549, and HepG2 cell lines with IC_{50} values as low as sub-micromole. These hydrides showed both in vitro and in vivo proliferation inhibition potency against breast cancer. Anwar et al. [9] produced derivative chalcones from hydroxy acetophenone and benzaldehyde in the presence of 50% KOH and found that it was active against breast cell line (T47D). A previous study synthesized several chalcones using the Microwave Assisted Organic Synthesis (MAOS) method through the aldol condensation process of aromatic ketone and aldehyde under alkaline conditions using NaOH catalyst [10]. The process has advantages such as short reaction times, pure reaction products, and higher yields. However, the test for breast cancer is still not reported yet.

In this study, chalcone was synthesized to be (E)-1-(4-chlorophenyl)-3-p-tolyprop-2-en-1-on (1), (E)-1-(4-chlorophenyl)-3-(4-tolyprophenyl) prop-2-en-1-on (2), (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on (3), and (E)-1-(3-bromophenyl)-3-(4-isopropyl phenyl) prop-2-en-1-on (4) using the Claisen-Schmidt condensation method by aldol condensation, in which new products of chalcone were synthesized from aldehyde and ketone aromatic by eco-friendly method (the method microwave radiation irradiated). The Vero cell was developed, the bioactivity was investigated using a cytotoxic test with the addition of Presto Blue TM, and absorbance measurements were performed using MCF-7 cell lines.

■ EXPERIMENTAL SECTION

Materials

The materials were 4-chloroacetophenone, 4-methylbenzhaldehide, 4-isoprophilbenzaldehide, 3-bromoacetophenon, 2-methyl acetophenone. 4-chlorobenzaldehide was used as aromatic ketone, and aldehyde was purchased from Merck (Darmstadt, Germany). KOH, HCl, *n*-hexane, ethyl acetate, methanol, ethanol, acetonitrile, distilled water, agar nutrient, broth nutrient, and KLT GF_{254} was obtained from Merck. In addition, some materials used were positive control (Doksorubisin), cell line cancer MCF-7, medium Rosewell Park memorial institute (RPMI) 1640 (GIBRO BRL), phosphate-buffered saline (PBS), prestoBlue™ cell viability reagent, fetal bovine serum (FBS), trypsin-EDTA, trypan Blue, and Cisplatin.

Procedure

Synthesis of (E)-1-(4-cholorophenil)-3-p-tollilprop-2-en-1-on (1)

The amount of 5 mmol (0.7730 g) of 4-chloroacetophenone as aromatic ketone and 5 mmol (0.6001 g) 4-methyl benzaldehyde as aromatic aldehyde was mixed in an Erlenmeyer glass and dissolved in 15 mL of ethanol. The mixture was added with 10 mL of KOH (5%) as a catalyst dropwise and homogenized using a magnetic stirrer. After that, it was irradiated using a microwave for 3 min at 800 °C and 700 W with an interval of 10 s to avoid evaporation. The reaction process was observed using TLC with *n*-hexane/ethyl acetate as the mobile phase. After that, the mixture was allowed for 20 h at room temperature to maximize the yield. 15 mL of distilled water was added and neutralized with 10% HCl. The precipitation formed was then filtered using a Buchner funnel, washed with cold *n*-hexane, and put in a vacuum oven [11].

Synthesis of (E)-1-(4-cholorophenil)-3-(4-isopropilfenil) prop-2-en-1-on (2)

The same procedure above was carried out for the synthesis of E-1-(4-chlorophenyl)-3-(4-isopropilfenil) prop-2-en-1-on (2) with a slight modification in which 5 mmol (0.7410 g) of 4-isopropilbenzaldehid was used as an aldehyde aromatic and the irradiation time was performed for 6 min at 80 °C and 700 W.

Synthesis of (E)-1-(3-bromofenil)-3-p-tolilprop-2-en-1-on (3)

The amount of 5 mmol (0.9952 g) 3-bromoacetophenon and 5 mmol (0.6001 g) of 4-methyl benzaldehyde were used as aromatic ketone and aromatic aldehyde, respectively. It was mixed in an Erlenmeyer and added with 15 mL of ethanol with 10 mL KOH (50%) as a catalyst. The mixture was irradiated in a microwave for 8 min at 80 °C and 700 W using an interval time of 10 s to avoid solvent evaporation. The following procedure was carried out as above.

Synthesis of (E)-1-(3-bromofenil)-3-(4-isopropilfenil) prop-2-en-1-on (4)

The compound of (E)-1-(3-bromofenil)-3-(4-isopropilfenil) prop-2-en-1-on was synthesized using 5 mmol (0.9952 g) 3-bromoacetophenon and 5 mmol (0.7410 g) of 4-isopropilbenzaldehid as aromatic ketone and aromatic aldehyde, respectively. It was mixed in an Erlenmeyer and added with 15 mL of ethanol with 10 mL KOH (50%) as a catalyst. The mixture was irradiated in a microwave for 8 min at 80 °C and 700 W using an interval time of 10 s to avoid solvent evaporation. The following procedure was carried out as above.

Purification of the compound

Compound 1-4 in the form of a yellow solid was dissolved with hot ethyl acetate in a dropwise fashion. The solution was filtered, and the filter was collected. It was then cooled to form a crystal, filtered using a Buchner funnel, washed using *n*-hexane, vacuumed, and characterized using TLC analysis.

Characterization

The eluent was prepared with a ratio of ethyl acetate:*n*-hexane (2:8, 1:9, 0.5:9.5, 0:10) and allowed to evaporate in a closed chamber to saturate the vapor. Compounds 1-4 were dissolved in ethyl acetate and spotted using a capillary tube at a distance of 0.7 cm from the lower edge of the silica gel plate GF254. The plate was inserted into the chamber, and the eluent was allowed to rise to the finish line. The plates were removed, and the strains were viewed with a UV lamp at wavelengths of 254 and 366 nm. The compound was pure if there was only one strain. TLC analysis with different eluent ratios was used to examine the purity of the compound, then the R_f value was determined. In addition, the structure of the compound 1-4 was analyzed using a UV/VIS spectrophotometer (UV-2610, China), FTIR (Nicolet 380, Thermo Scientific, Boston, USA), and ^{1}H-NMR (Agilent Technology, Santa Clara, CA, USA).

Cytotoxic analysis

Cytotoxicity as an anticancer in this study used cell lines MCF-7 and Vero cells which were developed at the University of Padjadjaran, Indonesia. Cell cultures used in 96 well plates were then incubated (at 37 °C and 5% CO_2 until the percentage of cells reached 70%), then the cells were treated with samples and incubated for 48 h at 37 °C and 5% CO_2 and added presto blue to the cell. The absorbance measurement used a Multimode reagent [12].

In this study, Presto Blue™ was used as a-based solution resazurin, which used live-cell reduction capabilities to measure cell proliferation quantitatively. Resazurin was added to the cells, and the absorbance was measured using a multimode reader. When cells were alive, they maintained a reducing environment in their cytosol. Upon entering living cells, Presto Blue™ was reduced to a red color, highly fluorescent resorufin. A healthy cell could be monitored by changes in fluorescent. Metabolically active cells were constantly changing Presto Blue™. On the other hand, non-viable cells could not reduce the indicator dye and therefore did not produce a signal change. The higher the intensity of the purple formed, the greater the number of living cells. In addition, the breast cancer cells used were MCF-7 cells derived from breast tissue of a 69-year woman with blood type O, Rh-positive, in the form of cells that were attached and could be grown in media.

■ RESULTS AND DISCUSSION

The final products of the synthesis of chalcone (C) 1-4 were yellow crystals with melting point of 156–157, 77–78, 131–132, and 66–68 °C, and the yield obtained was 1.1142 (87.03%), 0.9202 (64.80%), 0.9349 (62.32%), and 0.9075 g (55.32%), respectively. The R_f value for compound 1 at a ratio of 2:8; 1:9; 0.5:9.5; and 0:10 was 0.82, 0.77, 0.69 and 0.45, respectively. Using the same ratio, compound 2 had the R_f value 0.94, 0.87, 0.68, and 0.48. Meanwhile, compound 3 had 0.88, 0.78, 0.58, and 0.35, compound 4 was 0.88, 0.81, 0.54, and 0.20. A good Rf value was in the range from 0.2 to 0.8 [13].

The UV-Vis spectra of compounds 1-4 is shown in Fig. 1. Identification using a UV-Vis spectrophotometer provides information about the presence of conjugated bonds (double bonds), chromophore, and auxochrome of the synthesized chalcone. The results showed that compounds 1, 3, and 4 had three bands, at 286, 292, and 327 nm, while compound 2 had one band at 327 nm. The maximum absorption of the compounds was due to the π-π^* transition indicating the presence of a conjugated C=C chromophore [14]. Compounds 1-4 produced K bands or conjugated bands because they contained carbonyl compounds and produced B bands or benzenoid bands, indicating the presence of double bonds in the aromatic benzene ring.

FTIR spectra for compounds 1-4 are shown in Fig. 2. For compound 1, the presence of the C-Cl group, C=C group of benzene, and C=O ketone was seen at 812, 1512, and 1598 cm^{-1}, respectively. The band at 2916, 3028, and 3487 cm^{-1} are attributed to the presence of $-CH_3$, C–H of benzene, and overtone of C=O [15]. Compound 2 had an absorption at 817, 1512, and 1604 cm^{-1} indicating the presence of C-Cl group, C=C group of benzene, and the presence of C=O of ketones with a relatively constant number, intensity high, and generally free of distracting bands, making the band the most easily recognizable. The band at 2866 cm^{-1} is attributed to $-CH_3$ (asymmetric stretching), while symmetric stretching of $-CH_3$ and overtone of C=O were seen at 2962 and 3448 cm^{-1}, respectively [15]. FTIR spectra of compound 3 showed that absorption at 792, 1512, and 1604 cm^{-1} contributed to the presence of C–Br group, C=C group of benzene, and the presence of C=O of ketones, respectively. The presence of C–Br, C–H of benzene, and C=O were informed at 2362, 2960, and 3468 cm^{-1}, respectively [15].

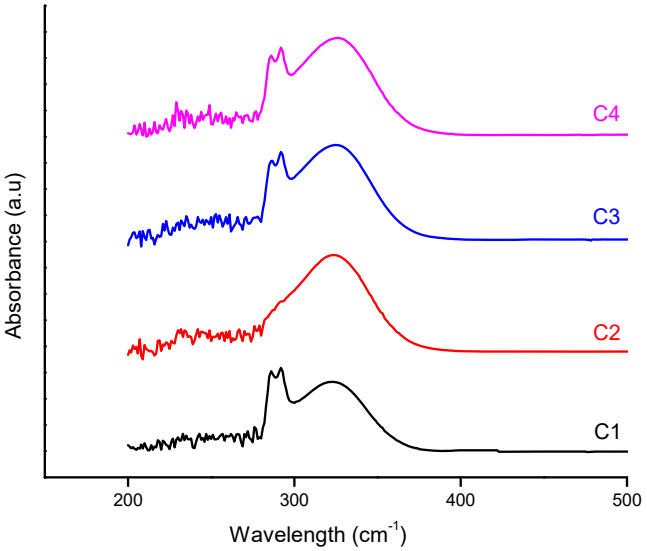


Fig 1. UV-Vis spectra of compounds: (C1) (E)-1-(4-chlorophenyl)-3-p-tolyprop-2-en-1-on, (C2) (E)-1-(4-chlorophenyl)-3-(4-tolyprophenyl) prop-2-en-1-on, (C3) (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on, and (C4) (E)-1-(3-bromophenyl)-3-(4-isopropylphenyl) prop-2-en-1-on

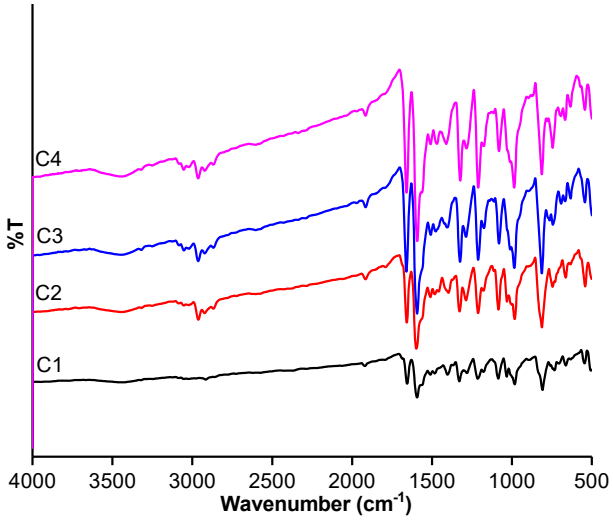


Fig 2. FTIR spectra for (C1) (E)-1-(3-bromofenil)-3-(4-isopropilfenil) prop-2-en-1-on, (C2) (E)-1-(3-bromofenil)-3-p-tolilprop-2-en-1-on, (C3) (E)-1-(4-cholorophenil)-3-(4-isopropilfenil) prop-2-en-1-on, and (C4) (E)-1-(4-cholorophenil)-3-p-tollilprop-2-en-1-on

Finally, compound 4 had the band at 790,1510, and 1604 cm^{-1}, corresponding to the presence of C-Br group, C=C group of benzene, and C=O of the ketone. The band at 2866, 2958, and 3485 cm^{-1}, respectively, attributed to the presence of $–CH_3$ (asymmetric), $–CH_3$ (symmetrical), and C=O [15-16].

Spectrum ^{1}H-NMR (CDCl3, 500 MHz) of common chalcone derivatives showed a typical chemical shift, namely the appearance of a double peak with a coupling constant of around 15–16 Hz due to the presence of protons in C_α (δ_H: 7.22–7.67 ppm) and C_β (δ_H: 7.49–7.94 ppm) to indicate trans configuration (E). The duplet peak at 6.5–8.2 ppm H chemical shift indicated H-aromatic resonance. Proton signal H 7.74 ppm (d, 1H) J = 16 Hz indicated H-signals H-aliphatic close to C-carbonyl, and H 7.89 ppm (d, 1H) J = 16 Hz corresponded (H_β = H-aliphatic far from C-carbonyl). The duplet signal at H 7.74 ppm was for one neighboring H atom. The signal at H 2.36 ppm (singlet) had the integrity of 3 protons, which especially characterized the presence of a methyl group ($–CH_3$) attached to an aromatic ring. H 1.5–2.5 ppm was a proton bound to the carbon next to the unsaturated bond. H 6.5–8 ppm, the protons in the aromatic ring (aryl protons) were strongly shielded by the orbitals on the ring and absorbed in this typical low field range [17-18]. The ^{1}H-NMR spectra of compounds 1-4 are shown in Fig. 3, and the measurement results are presented in Table 1.

MCF-7 Cancer Cell Cytotoxic Test with Presto Blue™

The cytotoxic test in this study used the colorimetric method due to color change caused by the oxidation-reduction reaction. Resazurin as a blue indicator is reduced to pink resorufin, and color change indicates cell activity. Cells that were still actively dividing carried out metabolic activities, resulting in enzymes derived from mitochondrial cell organelles and reduced resazurin.

The IC_{50} value was obtained from linear regression analysis between the log concentrations compound with percent inhibition of MCF-7 cell proliferation. The IC_{50} calculation data from compounds 1-4 are summarized in Table 2.

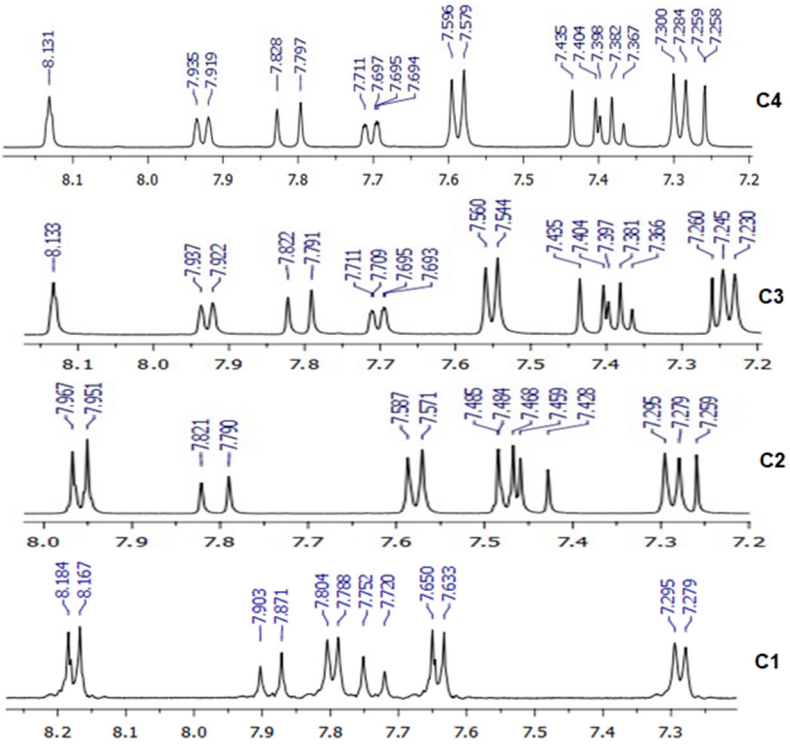
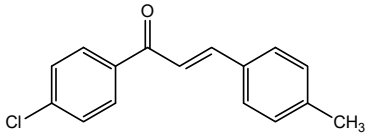
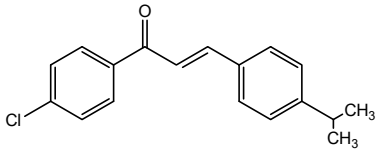
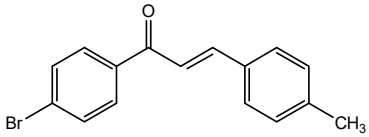
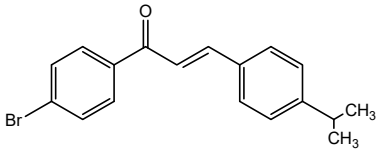


Fig 3. ^{1}H-NMR spectra of compounds (C1) (E)-1-(4-chlorophenyl)-3-p-tolyprop-2-en-1-on, (C2) (E)-1-(4-chlorophenyl)-3-(4-tolyprophenyl) prop-2-en-1-on, (C3) (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on, and (C4) (E)-1-(3-bromophenyl)-3-(4-isopropylphenyl) prop-2-en-1-on

Table 1. ^{1}H-NMR of compounds 1-4

Number atom	C1 δ_H ppm)	C2 δ_H (ppm)	C3 δ_H (ppm)	C4 δ_H (ppm)
1	-	-	-	-
2	7.80 (d, 1H) *J* = 8 Hz	7.58 (d, 1H) *J* = 8 Hz	7.55 (d, 1H) *J* = 8 Hz	7.59 (d, 1H) *J* = 8.5 Hz
3	7.29 (d, 1H) *J* = 8 Hz	7.29 (d, 1H) *J* = 8 Hz	7.24 (d, 1H) *J* = 7.5 Hz	7.29 (d, 1H) *J* = 8 Hz
4	-	-	-	-
5	7.29 (d, 1H) *J* = 8 Hz	7.29 (d, 1H) *J* = 8 Hz	7.24 (d, 1H) *J* = 7.5 Hz 7.55 (d, 1H) *J* = 8 Hz	7.29 (d, 1H) *J* = 8 Hz 7.59 (d, 1H) *J* = 8.5 Hz
6	7.80 (d, 1H) *J* = 8 Hz	7.58 (d, 1H) *J* = 8 Hz	7.42 (d, 1H) *J* = 715.5 Hz	7.42 (d, 1H) *J* = 15.5 Hz
Cα	7.74 (d, 1H) *J* = 16 Hz	7.44 (d, 1H) *J* = 15.5 Hz	7.81 (d, 1H) *J* = 15.5 Hz	7.81 (d, 1H) *J* = 15.5 Hz
Cβ	7.89 (d, 1H) *J* = 16 Hz	7.81 (d, 1H) *J* = 15.5 Hz	-	-
1'	-	-	8.13 (s, 1H)	8.13 (s, 1H)
2'	8.18 (d, 1H) *J* = 8.5 Hz	7.96 (d, 1H) *J* = 8 Hz	-	-
3'	7.64 (d, 1H) *J* = 8.5 Hz	7.48 (d, 1H) *J* = 8.25 Hz	7.70 (dd, 1H) *Ja* = 8 Hz, *Jb* = 1 Hz	7.70 (td, 1H) *Ja* = 8 Hz, *Jb* = 1 Hz
4'	-	-	7.38 (t,1H) *J* = 8 Hz	7.38 (t, 1H) *J* = 8 Hz 7.93 (d, 1H) *J* = 8 Hz
5'	7.64 (d, 1H) *J* = 8.5 Hz	7.48 (d, 1H) *J* = 8.25 Hz	7.93(d,1H) *J* = 7.5 Hz	1.27 (d, 6H) *J* = 7 Hz 2.95 (sep, 1H)
6'	8.18 (d, 1H) *J* = 8.5 Hz	7.96 (d, 1H) *J* = 8 Hz	2.40 (s, 3H)	
CH_3	2.36 (s. 3H)	1.27 (d, 6H) *J* = 7 Hz		
$CH(CH_3)_2$		2.950 (sep, 1H)		

Table 2. The structure and IC_{50} calculation of compounds 1-4

Compound	Structure	Linear regression equation	X= IC_{50} (ppm)*
1		y = -0.0002x + 0.587	1,484.75
2		y = -0.0114x + 0.7209	37.24
3		y = -0.0091x + 0.6673	42.22
4		y = -0.0228x + 0.8112	22.41

According to the National Cancer Institute [19], a compound has cytotoxic activity if IC_{50} 20 g/mL. Meanwhile, Meiyanto et al. [20] reported that the IC_{50} 100 g/mL in the compound showed anticancer activity that inhibited cell proliferation and was very potential as a Como prevention agent, as a compound to prevent the

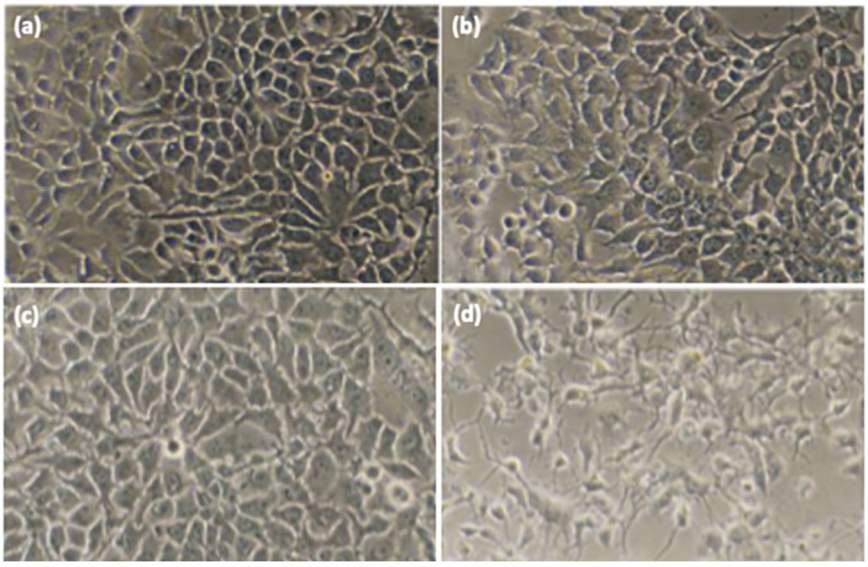


Fig 4. Morphology images of MCF7 for: (a) (E)-1-(4-chlorophenyl)-3-p-tolyprop-2-en-1-on; (b) (E)-1-(4-chlorophenyl)-3-(4-tolyprophenyl) prop-2-en-1-on; (c) (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on; and (d) (E)-1-(3-bromophenyl)-3-(4-isopropylphenyl) prop-2-en-1-on with a concentration of 7.81 µg/mL

carcinogenesis process of triggers cancer. Compounds 2-4 had an IC_{50} of 100 g/mL so that it was able to inhibit cell proliferation and was potential as a Como prevention agent. Meanwhile, compound 1 had no effect cytotoxic with LC_{50} of 1,484.75 g/mL.

In addition, IC_{50} indicates the concentration that produces resistance cell growth of 50% of the population and the potential of a compound as cytotoxic. The greater the IC_{50} value, the more the compound is non-toxic [21]. Cells with compound treatment showed morphological changes that lead to the characteristics of apoptotic cells in the form of shrinkage of cell dimensions (shrinkage) and cytoplasmic compaction, as well as extracellular matrix damage. Possible cytotoxic activity of compounds induces apoptosis and inhibits cell migration leading to antimetastatic [21]. The measurement of cell death for compounds 1-4 was indicated by changes in the morphology of the cell nucleus MCF-7 to be black, as seen in Fig. 4 (a–d).

Living cells appeared to have an elongated shape and were also attached to the base plate, while the dead cells were small, round, and floated. Activity test compound against MCF-7 breast cancer cells decreased the number of living cells with increasing concentration. The higher the concentration, the lower the number of living cells [22]. The biological activity of chalcone compounds in this study was influenced by the presence of α,β-unsaturated carbonyl groups and substituents bonded to the aromatic ring present in this compound.

■ CONCLUSION

The synthesized of chalcone into four compounds: (E)-1-(4-chlorophenyl)-3-p-tolyprop-2-en-1-on (1); (E)-1-(4-chlorophenyl)-3-(4-tolyprophenyl) prop-2-en-1-on (2); (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on (3); and (E)-1-(3-bromophenyl)-3-(4-isopropyl phenyl) prop-2-en-1-on (4) has been carried out and the yield of chalcone derivative from aromatic aldehyde compounds with halogen-substituted aromatic ketones by microwave radiation method are 87.03%, 64.80%, 62.32%, and 55.32%, respectively. Cytotoxic bioactivity of halogen-substituted chalcone analogue compounds 1-4 against MCF-7 breast cancer cells was 1,484.75 g/mL, 37.24 g/mL, 42.22 g/mL, 22.41 g/mL. The results shows that compound 2, 3 and 4 is potential as a Como prevention agent and able to inhibit cell proliferation.

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AUTHOR CONTRIBUTIONS

EMB conducted the experiment, JK and GH conducted the characterization, and EMB, JK, and MP wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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