

## Synthesis of Chalcone Derivatives and Their *in vitro* Anticancer Test against Breast (T47D) and Colon (WiDr) Cancer Cell Line

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Received July 24, 2017; Accepted January 24, 2018

### ABSTRACT

The synthesis of chalcone derivatives as target compounds and anticancer test against breast (T47D) and colon (WiDr) cell line had been performed. The synthesis was performed by Claisen-Schmidt condensation by using acetophenone and benzaldehyde derivatives. The anticancer activity test of chalcone derivatives was carried out by MTT assay against T47D and WiDr cell lines. The synthesis was started by reacting 4-hydroxyacetophenone and benzaldehyde derivatives such as p-anisaldehyde (chalcone 1 [(E)-4'-hydroxy-4-methoxychalcone]), veratraldehyde (chalcone 2 [(E)-4'-hydroxy-3,4-dimethoxychalcone]), 4-chlorobenzaldehyde (chalcone 3 [(E)-4'-hydroxy-4-chlorochalcone]) and 2,4-dihydroxyacetophenone with 4-chlorobenzaldehyde (chalcone 4 [(E)-2',4'-dihydroxy-4-chlorochalcone]) in methanol as solvent. The synthesis was carried out in alkaline condition (KOH) by stirring the mixture at room temperature for 48 h. The structures of products were identified by FTIR, GC-MS, <sup>1</sup>H- and <sup>13</sup>C-NMR spectrometers. The results showed that the chalcone derivatives (1-4) were yielded in 96; 97; 96; and 93%, respectively as yellow solid. The anticancer test indicated that the chalcone 4 was the most active towards T47D cell line with IC<sub>50</sub> of 42.66 µg/mL and the chalcone 3 was the most active against WiDr cell line with IC<sub>50</sub> of 20.42 µg/mL.

**Keywords:** chalcone derivatives; anticancer; breast cancer; colon cancer

### ABSTRAK

Telah berhasil disintesis senyawa turunan khalkon dan uji antikankernya terhadap sel kanker payudara (T47D) dan kolon (WiDr). Metode yang digunakan dalam sintesis ini adalah kondensasi Claisen-Schmidt menggunakan turunan benzaldehida dan asetofenon. Uji aktivitas antikanker terhadap sel kanker payudara (T47D) dan kolon (WiDr) dilakukan menggunakan metode MTT assay. Tahap sintesis dimulai dengan mereaksikan 4-hidroksiasetofenon dengan turunan benzaldehida, seperti: p-anisaldehyda (khalkon 1 [(E)-4'-hidroksi-4-metoksikhalkon]), veratraldehyda (Khalkon 2 [(E)-4'-hidroksi-3,4-dimetoksikhalkon]), dan 4-klorobenzaldehida (khalkon 3 [(E)-4'-dihidroksi-4-klorokhalkon]) dan 2,4-dihidroksiasetofenon dengan 4-klorobenzaldehida (khalkon 4 [(E)-2',4'-dihidroksi-4-klorokhalkon]) dalam pelarut metanol. Sintesis dilakukan dalam suasana basa menggunakan KOH dan campuran diaduk selama 48 jam pada suhu kamar. Kebenaran struktur produk dielusidasi menggunakan spektrometer FTIR, GC-MS, <sup>1</sup>H- and <sup>13</sup>C-NMR. Hasil sintesis khalkon berupa padatan berwarna kuning dengan rendemen khalkon 1-4 masing-masing 96; 97; 96; dan 93%. Uji antikanker menunjukkan bahwa khalkon 4 paling aktif terhadap sel antikanker T47D dengan IC<sub>50</sub> 42,66 µg/mL dan khalkon 3 paling aktif terhadap sel antikanker WiDr dengan IC<sub>50</sub> 20,42 µg/mL.

**Kata Kunci:** turunan khalkon; antikanker; kanker payudara; kanker kolon

### INTRODUCTION

Cancer is a disease characterized by rapid cell growth and uncontrolled [1]. Cancer became the second disease that causes death worldwide after cardiovascular diseases [2]. Based on the World Health Organization (WHO) data, in 2012 there were

approximately 14 million new cases of cancer and 8.8 million deaths in 2015 are caused by cancer [3]. In Indonesia, according to the Data and Information Centre, Ministry of Health in 2013, cancer patients reached 347.792 people [4].

The method of cancer treatment that used today is radiation and surgery. Treatment with radiation is

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able to kill local cancer, but it can also kill normal cells around [5]. Chemotherapy can be used as an alternative cancer treatment, but Ranjit et al. [6] said that some chemotherapy drugs give side effects such as marrow depression and alopecia. Therefore, it is necessary to develop a safer chemopreventive agent so that the side effects are relatively small compared with surgery and radiation [7]. One of the compounds that have potential as a chemoprevention agent is chalcone.

Li et al. [8] succeed to isolate the chalcone from 8.5 kg of *Desmodium renifolium* and obtained 25.9 mg (0.0003%) isolates of prenylchalcone. This chalcone has excellent pharmacological activity as anticancer against leukemia (NB4), lung (A549) and breast (MCF-7) cancer cell with IC<sub>50</sub> 3.40; 3.196; and 3.40 µg/mL respectively. However, the isolation methods are less due to the little amount of the isolate. According to Patil et al. [9] and Loudon [10], chalcone can be synthesized by Claisen-Schmidt condensation using acetophenone and benzaldehyde derivatives using acid or base catalysts.

An important factor influencing the anticancer activity of chalcones contained in C<sub>α</sub> and C<sub>β</sub> unsaturated bond. Besides that, the presence of hydroxy, methoxy, and prenyl in the ring A is known to inhibit the growth of cancer cells by attacking the cell nucleus area, while the presence of the hydroxy group and methoxy in ring B can affect an active part of the cell, so that the cell can not have cleavage. However, the addition of the hydroxy group at position C6' ring A does not indicate specific activity and can reduce cytotoxicity of chalcones [11]. According to Mai et al. [12], the chloro and methoxy groups in ring B can contribute to the anti-proliferation activity of chalcones. Suwito et al. [13] compared the effect of the position of the methoxy on C2, C3, and C4 to the anticancer activity of chalcones, this study showed that the methoxy group is most active as anticancer on the C4 position of ring B chalcones. Zhang et al. [14] also compared the effect of halogen groups and its position on the C2, C3, and C4 to cell inhibitory activity. The results showed that the chloro group on C4 position have the best cytotoxic activity.

A lot of chalcone have been synthesized by the previous researcher especially for the methoxylated chalcone in ring B [15-16]. However, the presence of the chloro groups at ring B of chalcone and the effect of chloro group towards T47D and WiDr cancer cell lines has not been reported yet. Therefore, in this research, we will report the synthesis of chalcone with chloro substituents and also the cytotoxicity test of those chalcones by MTT method towards cancer cell lines T47D (breast cancer) and WiDr (colon cancer).

## EXPERIMENTAL SECTION

### Materials

The materials used were obtained from Merck with p.a. quality, i.e., *p*-anisaldehyde, veratraldehyde, 4-hydroxyacetophenone, 2,4-dihydroxyacetophenone, methanol, potassium hydroxide, *n*-hexane, ethyl acetate, dimethyl sulfoxide (DMSO), iodine and ethanol, while *p*-chlorobenzaldehyde was obtained from Sigma-Aldrich. Thin layer chromatography was performed using aluminum plates (Merck) were coated with silica gel 60 F254 (20 x 20 cm), and column chromatography was carried out using Merck silica gel 60 (0.063–0.200 mm). Materials for *in vitro* anticancer test were breast cancer cells (T47D), colon cancer cells (WiDr) normal cells (Vero), medium Dulbecco's Modified Eagle's Medium (DMEM, Sigma), Fetal Bovine Serum (FBS) 10% (w/v) (Sigma), dimethyl sulfoxide, phosphate buffer solution (PBS), solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT), sodium dodecyl sulfate (SDS) 10% (w/v), and HCl 0.1 N.

### Instrumentation

All melting points were determined by digital melting point apparatus (electrothermal-9100), and are uncorrected. Infrared spectra were recorded with Shimadzu Prestige-21 FT-IR spectrometer using KBr discs. Mass spectra were recorded on a Shimadzu GC-MS QP-2010S (EI). <sup>1</sup>H and <sup>13</sup>C-NMR spectra were obtained on a JEOL JNM-ECA 500 (500 MHz) spectrometer using internal standard TMS and deuterated chloroform and methanol as solvents.

The *in vitro* anticancer test were performed using microwell plate 96 (Iwaki), autoclave, sterile conical tubes, blue and yellow micropipette tips, micropipette 2–20 mL (Nichipet EX H32014262), micropipette 20–100 mL and 200–1000 mL (Gilson X57732D), ELISA microplate reader (BIO-RAD Benchmark), 5% CO<sub>2</sub> incubator (NAPCO models 6200, Heraeus), hemocytometer (Neubauer), Laminary Air Flow (LABCONCO Purifier Class II Biosafety cabinet), and an inverted microscope (Olympus CKX41-1X2SL).

### Procedure

#### Synthesis of chalcones 1-4

**(E)-4'-Hydroxy-4-methoxychalcone (Chalcone 1).** Synthesis chalcone was performed according to the

procedure by Hsieh et al. [17] with some modifications. A total of 0.41 g (3 mmol, 1 equiv.) 4-hydroxyacetophenone was put into the round-bottom flask, then 10 mL of absolute methanol and 5 mL of KOH with various concentration (30, 40, 50 and 60% w/v in water or 9, 12, 15 and 18 equiv., respectively) was added. Next, 0.41 g (3 mmol, 1 equiv.) *p*-anisaldehyde was added, and the mixture was stirred for 48 h at room temperature. The reaction was monitored by thin layer chromatography. This mixture was poured into ice-cold water followed by neutralization with HCl 10% to produce yellow precipitates. The resulting precipitate was filtered off, washed and dried and purify via recrystallization with absolute methanol to give chalcone **1** (0.73 g, 96%) as a yellow solid. mp. 186–187 °C. IR (cm<sup>-1</sup>): 3448 (O–H), 3132 (C<sub>sp2</sub>-H), 1643 (C=O), 1512 and 1566 (C=C Ar), 1033 (C–O), 987 (C=C trans). <sup>1</sup>H-NMR spectrum (500 MHz, CDCl<sub>3</sub>, ppm): δ 3.86 (s, 3H, OCH<sub>3</sub>), 5.58 (s, 1H, OH), 6.93 (m, 4H, ArH), 7.41 (d, *J* = 15.50 Hz, 1H, H<sub>α</sub>), 7.60 (d, *J* = 9.00 Hz, 2H, ArH), 7.77 (d, *J* = 15.50 Hz, 1H, H<sub>β</sub>), 7.99 (d, *J* = 8.50 Hz, 2H, ArH). <sup>13</sup>C-NMR spectrum (125 MHz, CDCl<sub>3</sub>, ppm): δ 55.4 (OCH<sub>3</sub>), 114.4 (2 ArCH), 115.4 (2 ArCH), 119.5 (C<sub>α</sub>), 127.8, 130.1 (2 ArCH), 131.0 (2 ArCH), 131.6, 144.0 (C<sub>β</sub>) 159.7, 161.5 (ArC), 188.8 (CO). Mass Spectrum (EI): *m/z* 254 (M, 100%), 239 (30), 211(30), 121(80), 65 (90), 39 (90).

**(E)-4'-Hydroxy-3,4-dimethoxychalcone (Chalcone 2).** This was prepared as described for the Chalcone **1** from a solution of 4-hydroxyacetophenone (0.41 g, 3 mmol, 1 equiv.), veratraldehyde (0.5 g, 3 mmol, 1 equiv.) in methanol and 50% KOH (15 equiv.) to give chalcone **2** (0.82 g, 97%) as a yellow solid. mp. 197–199 °C. IR (cm<sup>-1</sup>): 3448 (O–H), 3147 (C<sub>sp2</sub>-H), 1643 (C=O), 1512 and 1589 (C=C Ar), 1026 (C–O), 987 (C=C trans). <sup>1</sup>H-NMR spectrum (500 MHz, CDCl<sub>3</sub>, ppm): δ 3.94 (s, 3H, OCH<sub>3</sub>), 3.96 (s, 3H, OCH<sub>3</sub>), 5.91 (s, 1H, OH), 6.90 (d, *J* = 8.00 Hz, 1H, ArH), 6.94 (d, *J* = 9.50 Hz, 2H, ArH), 7.16 (d, *J* = 2.00 Hz, 1H, ArH), 7.23 (dd, *J* = 2.00 and 8.00 Hz, 1H, ArH), 7.39 (d, *J* = 15.50 Hz, 1H, H<sub>α</sub>), 7.75 (d, *J* = 15.50 Hz, 1H, H<sub>β</sub>) and 8.00 (d, *J* = 9.50 Hz, 2H, ArH). <sup>13</sup>C-NMR spectrum (125 MHz, CDCl<sub>3</sub>, ppm): δ 55.9, 56.0 (OCH<sub>3</sub>), 110.1, 111.1, 115.4 (2 ArCH), 119.7 (C<sub>α</sub>), 123.1, 128.0, 131.1 (2 ArCH), 131.4, 144.4 (C<sub>β</sub>), 149.2, 151.3, 159.9 (ArC) and 188.9 (CO). Mass Spectrum (EI): *m/z* 284 (M, 30%), 269 (15), 241 (15), 191 (10), 121 (70), 93 (50), 65 (100).

**(E)-4'-Hydroxy-4-chlorochalcone (Chalcone 3).** This was prepared as described for the Chalcone **1** from a solution of 4-hydroxyacetophenone (0.41 g, 3 mmol, 1 equiv.), *p*-chlorobenzaldehyde (0.42 g, 3 mmol, 1 equiv.) in methanol and 50% KOH (15 equiv.) to give chalcone **3** (0.75 g, 96%) as a yellow solid. mp. 188–190 °C. IR (cm<sup>-1</sup>): 3387 (O–H), 3093 (C<sub>sp2</sub>-H), 1643 (C=O), 1543 and 1597 (C=C aromatic), 1033 (C–O), 979 (C=C trans). <sup>1</sup>H-

NMR spectrum (500 MHz, CDCl<sub>3</sub>, ppm): δ 6.89 (d, *J* = 9.10 Hz, 2H, ArH), 7.43 (d, *J* = 8.45 Hz, 2H, ArH), 7.69 (d, *J* = 15.60 Hz, 1H, H<sub>α</sub>), 7.72 (d, *J* = 8.45 Hz, 2H, ArH), 7.76 (d, *J* = 15.60 Hz, 1H, H<sub>β</sub>); and 8.02 (d, *J* = 9.10 Hz, 2H, ArH). <sup>13</sup>C-NMR spectrum (125 MHz, CDCl<sub>3</sub>, ppm): δ 116.6 (2 ArCH), 123.8 (C<sub>α</sub>), 130.3 (2 ArCH), 130.9, 131.1 (2 ArCH), 132.6 (2 ArCH), 135.4, 137.3, 164.2 (ArC), 143.6 (C<sub>β</sub>) and 190.5 (CO). Mass Spectrum (EI): *m/z* 260 (M+2, <sup>37</sup>Cl, 20%), 258 (M, <sup>35</sup>Cl, 60), 223 (30), 165 (25), 146 (15), 121 (100), 93 (40), 65 (70).

**(E)-2',4'-Dihydroxy-4-chlorochalcone (Chalcone 4).** This was prepared as described for the Chalcone **1** from a solution of 2,4-dihydroxyacetophenone (0.46 g, 3 mmol, 1 equiv.), *p*-chlorobenzaldehyde (0.42 g, 3 mmol, 1 equiv.) in methanol and 50% KOH (15 equiv.) to give chalcone **4** (0.76 g, 93%) as a dark yellow solid. m.p. 204–206 °C. IR (cm<sup>-1</sup>): 3271 (O–H), 3047 (C<sub>sp2</sub>-H), 1635 (C=O), 1512 and 1589 (C=C aromatic), 1095 (C–O), 972 (C=C trans). <sup>1</sup>H-NMR spectrum (500 MHz, CDCl<sub>3</sub>, ppm): δ 6.30 (d, *J* = 2.60 Hz, 1H, ArH), 6.42 (dd, *J* = 2.60 and 9.10 Hz, 1H, ArH), 7.44 (d, *J* = 8.40 Hz, 2H, ArH), 7.69 (d, *J* = 15.70 Hz, 1H, H<sub>α</sub>), 7.75 (d, *J* = 8.40 Hz, 2H, ArH), 7.82 (d, *J* = 15.70 Hz, 1H, H<sub>β</sub>) and 8.00 (d, *J* = 8.40 Hz, 1H, ArH). <sup>13</sup>C-NMR spectrum (125 MHz, CDCl<sub>3</sub>, ppm): δ 103.9, 109.4, 114.7, 122.7 (C<sub>α</sub>), 130.3 (2 ArCH), 131.2 (2 ArCH), 133.7, 135.2, 137.4, 143.6 (C<sub>β</sub>), 166.9, 167.8 (ArC) and 193.2 (CO). Mass Spectrum (EI): *m/z* 276 (M+2, <sup>37</sup>Cl, 15%), 274 (M, <sup>35</sup>Cl, 50), 163 (50), 136 (100), 108 (95), 80 (40), 51 (50).

#### **In vitro anticancer test of chalcones 1-4 against breast (T47D) and colon (WiDr) cell line**

A total of 100 μL of cell suspension in DMEM containing 10<sup>6</sup> cells was inserted into each of the microwell plate 96 and incubated in an incubator at 37 °C, 5% CO<sub>2</sub> for 24 h. A total of 100 μL of test compounds solution of each concentration was put in a cell suspension in wells that had been incubated previously. As control of the media and cell control, added 100 μL of DMEM and cancer cells in wells. A culture that has been given the test compounds was incubated for 24 h at 37 °C, 5% CO<sub>2</sub> in the incubator. After the incubation process was completed, the culture medium was removed and washed with PBS, then 100 μL of MTT (1 mL of MTT and 9.5 mL of DMEM) was added to wells. Next, microwell plate was incubated again at 37 °C, 5% CO<sub>2</sub> in an incubator for 4 h. After that, 100 mL of 10% SDS stopper in 0.1 N HCl was added into each of the wells (to dissolve the purple formazan), then the wells were wrapped tightly with paper and left at room temperature overnight. Absorbance readings were performed by ELISA microplate reader at a wavelength of 595 nm. Cell viability can be calculated using eq. 1.

$$\% \text{ of cell growth} = \frac{(a-b)}{(c-b)} \times 100\% \quad (1)$$

where, a = absorbance of the sample; b = absorbance media controls; and c = absorbance of cell controls

## RESULT AND DISCUSSION

### Synthesis of Chalcones 1-4

The Claisen Schmidt condensation of 4-hydroxyacetophenone with benzaldehyde derivative with various substituents has been done by adopting the procedure from Hsieh et al. [17] with slight modifications and the synthetic scheme of chalcone 1-4 was presented in Fig. 1.

Chalcone 1 has been synthesized by Sultan *et al.* [15] under an alkaline condition with the same base (KOH) and yielded the product in 32%, while Chalcone B has also been prepared by Narender and Reddy [18] in 90% yield by using a catalyst BF<sub>3</sub>-Et<sub>2</sub>O. To achieve the maximum yield by the use of a simple base such as KOH, in this experiment we varied the amount of KOH, and the result was shown in Table 1. The highest yield resulted from 60% of KOH, but the yield of using 50% KOH was not significantly different from 60%. Therefore for all of the chalcone synthesis, we used the same concentration of KOH (50%). The highest concentration of the base possibly needed due to the acidic OH of 4-hydroxyacetophenone.

With the optimum condition, chalcone 2 was produced by reacting 4-hydroxyacetophenone with veratraldehyde and yielded a yellow solid in 97%. The structure of chalcone 2 was confirmed by spectral data (IR, NMR, and MS) [18].

Chalcone 3 and 4 was synthesized from 4-chlorobenzaldehyde with 4-hydroxyacetophenone and 2,4-dihydroxyacetophenone and yielded in 96 and 93%, respectively. The IR spectra of both chalcones 3 and 4 displayed bands at 3387–3271 cm<sup>-1</sup> due to O–H stretching and the carbonyl group also shifted from 1674 to 1635 cm<sup>-1</sup>, characteristic for conjugated carbonyl

vibration [19]. The product also showed disappearing peaks at 2746, and 2846 cm<sup>-1</sup> (CH-aldehyde) and a new peak appeared at 987–972 cm<sup>-1</sup> for CH trans-bend [20] in FTIR. The mass spectrum of chalcone 3 and 4 revealed a molecular ion at m/z 260 and 276 for both M+2 (<sup>37</sup>Cl), respectively. Whereas the <sup>13</sup>C-NMR spectrum clearly showed the presence of an alkene carbon (C<sub>α</sub> and C<sub>β</sub>) resonating at 123 and 144 ppm and carbonyl at 190 and 193 ppm for chalcone 3 and 4. The formation of trans alkenes were also clarified by <sup>1</sup>H-NMR with the appearing of two doublet peaks at 7.69 and 7.76 ppm (*J* = 15.6 Hz) for chalcone 3 and at 7.69 and 7.82 ppm (*J* = 15.7 Hz) for chalcone 4 [21]. Even though the hydroxy peaks were not observed in <sup>1</sup>H NMR spectra, but all of this assignments were consistent with the formation of chalcone 3 and 4.

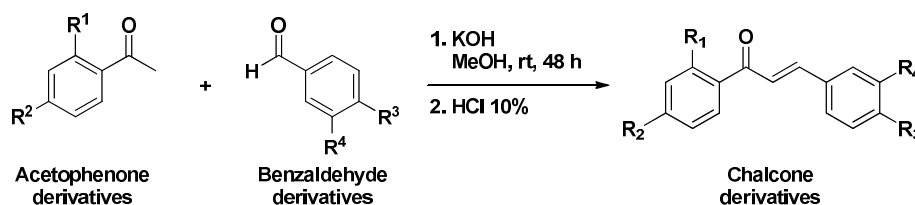
The results showed that the method was efficient and simple. In addition, it was also general since it worked very well with various substrates with different type and position of substituents.

### In Vitro anticancer Test of Chalcones 1-4 Against breast (T47D) and colon (WiDr) cell line

Chalcone 1-4 was tested as an anticancer agent by MTT method. Compounds test could be said to be active in the inhibition of cell growth if they had IC<sub>50</sub> values less than 20 μg/mL, IC<sub>50</sub> between 20–100 μg/mL had moderate activity, and IC<sub>50</sub> values greater than 100 μg/mL is not active in the inhibition of cell growth [22]. Based on this reference, it was known that chalcone 1-4 had moderate activity in the inhibition of cancer cell T47D and WiDr (Table 2).

**Table 1.** KOH Equivalent of chalcone A synthesis

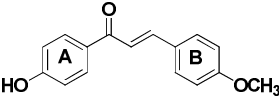
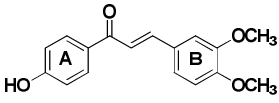
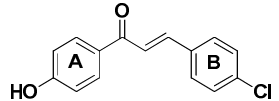
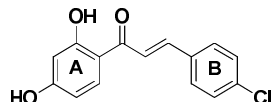
Entry	KOH (Equiv.)	Yield (%)
1	9	82
2	12	90
3	15	96
4	18	97



Chalcone 1: R<sub>1</sub> = H; R<sub>2</sub> = OH; R<sub>3</sub> = OCH<sub>3</sub>; R<sub>4</sub> = H, 96%  
 Chalcone 2: R<sub>1</sub> = H; R<sub>2</sub> = OH; R<sub>3</sub> = R<sub>4</sub> = OCH<sub>3</sub>, 97%  
 Chalcone 3: R<sub>1</sub> = H; R<sub>2</sub> = OH; R<sub>3</sub> = Cl; R<sub>4</sub> = H, 96%  
 Chalcone 4: R<sub>1</sub> = R<sub>2</sub> = OH; R<sub>3</sub> = Cl; R<sub>4</sub> = H, 93%

**Fig 1.** Synthesis of chalcones 1-4

**Table 2.** IC<sub>50</sub> values and selectivity index of chalcone (1-4) against cancer cells

Compound	IC <sub>50</sub> (µg/mL)			Selectivity index	
	T47D	WiDr	Vero	T47D	WiDr
 Chalcone 1	72.44	44.67	165.96	2.29	3.72
 Chalcone 2	44.67	29.51	2.69 x 10 <sup>6</sup>	60.22	91.115
 Chalcone 3	57.70	20.42	123.03	2.13	6.02
 Chalcone 4	42.66	31.61	61.66	1.45	1.95

From Table 2, it can be seen that the IC<sub>50</sub> value of Chalcone 2 (44.67 µg/mL) is smaller than that of chalcone 1 (72.44 µg/mL). It means that the addition of methoxy at ring B at position 3 could increase the inhibition of T47D cancer cells. This result is in accordance with Mai et al. [12] which states that the presence of methoxy groups may affect the activity of cancer cell inhibition. It was also observed that the chloro substituents on chalcone 3 with IC<sub>50</sub> 57.70 µg/mL showed a better inhibitory activity than methoxy group on chalcone 1 with IC<sub>50</sub> 72.44 µg/mL. This result is similar to the research conducted by Ketabforoos et al. [23] which states that chloro substituents are more active in inhibition of breast cancer cells. Furthermore, the addition of hydroxyl group on ring A at position 2 also increases the inhibitory activity of T47D cancer cells. This can be seen from the fact that the IC<sub>50</sub> of chalcone 4 (42.66 µg/mL) is lower than that of chalcone 3 (57.70 µg/mL). Based on this result, the chalcone 4 has the best inhibitory activity towards the T47D cell, but this compound is not selective due to the high IC<sub>50</sub> value (61.66 µg/mL) against normal cell (Vero). Therefore, it can be concluded that the most selective chalcone against T47D cancer cells is chalcone 2 with a selectivity index of 60.22.

Anticancer assay of chalcone 1 and 2 towards colon cancer cell (WiDr) showed that the addition of methoxy group at ring B at position 3 increased the inhibition activity. The IC<sub>50</sub> of chalcone 1 (44.67 µg/mL) decreased the IC<sub>50</sub> value into 29.51 µg/mL on chalcone 2. In addition, the chloro substituent on ring B on chalcone 3 also increased the inhibition activity against cancer cell WiDr compared with the methoxy group

(chalcone 2) [23]. However, the effect of a hydroxy group towards WiDr cancer cells is different from T47D cancer cells. The hydroxy group on ring A at position 2 (chalcone 4) were found reducing the activity when applied to WiDr cancer cells (chalcone 3) [11]. This is probably due to the ability of the hydroxyl group to form the hydrogen bonding and prevents the interaction with the cancer cell line. Chalcone 3 was the most active against an anticancer colon cell (WiDr) but less selective. Therefore, the most active and selective chalcone towards WiDr cancer cell is chalcone 2 with a selectivity index of 91.12.

## CONCLUSION

Chalcones 1-4 was successfully synthesized from hydroxyacetophenone and benzaldehyde derivatives in the presence of 50% KOH in high yields, i.e., 96; 97; 96; and 93%, respectively. Chalcones 1-4 has a moderate activity towards breast (T47D) and colon (WiDr) cancer cell lines. Based on the IC<sub>50</sub> values and the selectivity index, it can be concluded that the most active compound towards breast cell line (T47D) was chalcone 4, while the most active compound against a colon cancer cells (WiDr) was chalcone 3.

## ACKNOWLEDGEMENT

We would like to thanks to Ministry of Research, Technology and Higher Education of the Republic of Indonesia for financial support via PUPT-UGM 2016 Research scheme (Contract No. 015/SP2H/LT/DRPM/II/2016, 17 February 2016).

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