

## RESEARCH ARTICLE

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## Cytotoxic Activity of Methoxy-4' amino Chalcone Derivatives Against Leukemia Cell Lines

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**Background:** Chemotherapy is a common treatment for leukemia as well as in other cancer treatment. The lack of tumor selectivity and development of multi-drug resistance by chemotherapy caused the development of new strategy in cancer treatment become a pressing need. This study was performed to evaluate the anticancer activity and selectivity of seven derivatives of chalcones against K562 and HL-60 leukemia cell lines.

**Materials and Methods:** The cytotoxicity of chalcone's seven derivatives (compound 1-7) was tested by using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) method. The percentage of cell mortality data was calculated then the  $IC_{50}$  was analyzed using probit analysis (SPSS 17). The selectivity index (SI) then calculated from  $IC_{50}$  ratio of normal lymphocyte cells and cancerous cells line (HL-60 and K562).

**Results:** The  $IC_{50}$  of almost all seven tested compounds were lower in HL-60 cell lines (ranged from 1.57 to 9.63  $\mu$ g/mL) than K562 cell lines (ranged from 5.87 to 52.56  $\mu$ g/mL), except for Compound 7 ( $5.87 \pm 0.15$   $\mu$ g/mL). The number and position of methoxy groups in chalcone derivatives influenced the anticancer and cancer selectivity of chalcone derivatives.

**Conclusion:** The results revealed that the number and position of methoxy groups in chalcone derivatives influenced the anticancer and cancer selectivity of chalcone derivatives.

**Keywords:** anticancer, chalcone derivatives, methoxy-4'-amino chalcone, leukemia, cytotoxic, selectivity

### Introduction

Leukemia is cancer of the white blood cells characterized by widespread, rapid, and disorderly proliferation of leukocytes and their precursor as well as by the presence of immature leukocytes in blood often in very large number.<sup>1,2</sup> Although leukemia is a rare disease, however it exceeds a cause of

death from many of the acute communicable diseases due to its fatal character.<sup>2</sup> In 2012, leukemia suffered by approximately 352,000 people around the world and caused 265,000 death.<sup>3</sup>

Chemotherapy is common for the treatment of leukemia as well as other cancer.<sup>4</sup> Nowadays, chemotherapy is one of the most effective and potent strategies to treat

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cancer. However, the use of anticancers in the chemotherapy is limited. All the anticancer currently available have severe side effects on normal cells due to their nonspecificity. In addition, the resistance of cancer cells to the anticancer remains a significant impediment to successful chemotherapy.<sup>5</sup> Therefore, the need to discover and develop new anticancers with specific target and novel mechanisms is urgent.

Natural products are important and valuable resources for anticancer discovery and development. Natural products demonstrate important structural diversity and offer a wide variety of novel compounds with novel anticancer mechanisms.<sup>6,7</sup> Vincristine, irinotecan, paclitaxel, actinomycin D, mitomycin C, and doxorubicin are classic examples of natural-derived anticancer that used in clinic. During the last few years, natural-product-based drug discovery is increasing based on new technologies, such as combinatorial synthesis and high-throughput screening, and their associated approaches.<sup>6</sup>

Chalcones are precursor of flavonoids that widely distributed in plants and one of potential natural compounds to develop as anticancer. A number of chalcones have been isolated or synthesized and evaluated for their cytotoxicity on cancer cell lines culture and their anticancer activity on cancer animal model.<sup>8,9</sup> Furthermore, the molecular mechanisms of anticancer action of chalcones have also investigated. The chalcones exhibited anticancer activity through multiple mechanisms included cell cycle disruption, angiogenesis inhibition, tubulin polymerization inhibition, apoptosis induction and blockade of nuclear factor-kappa B (NF- $\kappa$ B) signaling pathway.<sup>9-13</sup>

In our previous study, some derivatives of methoxy amino chalcone have been synthesized and evaluated for their cytotoxicity on breast cancer cell culture.<sup>14,15</sup> In this study we evaluated the cytotoxicity of the methoxy amino chalcone derivatives on (human acute promyelocytic leukemia (HL-60) and human chronic myelogenous leukemia (K562) cells line. The cytotoxicity of these derivatives on normal lymphocyte cells was also evaluated in order to determine their selectivity index (SI).

## Materials and methods

### Tested Compounds

The methoxy amino chalcone derivatives were synthesized by Drs. Hery Suwito in the Department of Chemistry, Faculty of Science and Technology, Airlangga University,

Surabaya, Indonesia. The chemical structure of the methoxy amino chalcone derivatives are showed in Table 1.

### Cell Culture

The HL-60 cell line, K562 cell line and lymphocytes were used for cytotoxic screening of the tested compounds. The HL-60 and K562 cell lines were obtained from Stem Cells and Cancer Institute (SCI) Jakarta, Indonesia. The cell lines were maintained in Iscove's Modified Dulbecco's (IMD) medium supplemented by 10% fetal bovine serum (FBS), and 2% penicillin-streptomycin. The cell lines were maintained at 37°C in a 5% CO<sub>2</sub> atmosphere with 95% humidity. Lymphocytes were isolated from whole blood using Histopaque (Sigma) according to the manufacturer's instructions. The lymphocytes culture were then maintained in RPMI1640 medium supplemented with 1% non-essential amino acids, 1% L-glutamine, 100 U/mL penicillin, 10 mg/mL streptomycin and 10% heat inactivated fetal calf serum at 37°C in a 5% CO<sub>2</sub> atmosphere with 95% humidity.

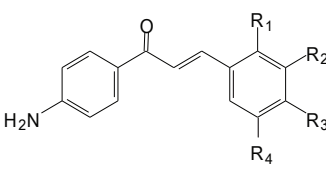
### Cytotoxic Assay

Cytotoxic activity of the tested compounds on the treated cells were determined using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethylthio)phenyl-2-(4-sulfophenyl)-2H-tetrazolium (MTS) colorimetric assay developed by Yoon, *et al.*,<sup>16</sup> and Khan, *et al.*,<sup>17</sup> after modification. Around 90  $\mu$ L of the treated cells culture at density of 10<sup>4</sup> cells/well were distributed in 96-wells plates. Ten microlitres of the tested compounds at various concentration (0.2-100  $\mu$ g/mL) then added to each well, except for the negative control the well was added with the culture medium and for the positive control the well was added with imatinib or accutane. The plate then incubated for 24 h at 37°C in a 5% CO<sub>2</sub>. Following the incubation, the mixture further added with 20  $\mu$ L of MTS, incubated for 4 h at 37°C in 5% of CO<sub>2</sub>. The absorbance then measured at  $\lambda$  of 490 nm using a microplate reader.<sup>18</sup> The cell growth inhibition was determined with the equation:

$$\% \text{ of cell growth inhibition} = \frac{\text{OD of control cell} - \text{OD of treated cell}}{\text{OD of control cell}} \times 100\%$$

For this MTS method, IC<sub>50</sub> (concentration that inhibit 50% cell growth) values were determined using probit analysis. The IC<sub>50</sub> values was obtained from three independent experiments. Furthermore, selectivity index (SI) was determined from the IC<sub>50</sub> ratio of lymphocytes normal

**Table 1. Chemical structure of different methoxy amino chalcone derivatives.**

Methoxy amino chalcone structure					
					
Compound	Chemical name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
1	(E)-1-(4-aminophenyl)-3-(2-methoxyphenyl)-prop-2-en-1-one	OCH <sub>3</sub>	H	H	H
2	(E)-1-(4-aminophenyl)-3-(3-methoxyphenyl)-prop-2-en-1-one	H	OCH <sub>3</sub>	H	H
3	(E)-1-(4-aminophenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one	H	H	OCH <sub>3</sub>	H
4	(E)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)-prop-2-en-1-one	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H
5	(E)-1-(4-aminophenyl)-3-(2,4-dimethoxyphenyl)-prop-2-en-1-one	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H
6	(E)-1-(4-aminophenyl)-3-(2,5-dimethoxyphenyl)-prop-2-en-1-one	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>
7	(E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one	H	H	H	H

and cancerous cells (K562 and HL-60). The SI value demonstrates selectivity of the tested compound to the cell lines tested. Samples with SI value greater than 2 were considered to have good selectivity.<sup>19</sup>

### Statistical Analysis

Data of the IC<sub>50</sub> values and SI were presented as mean  $\pm$  standard deviation (SD) and were analyzed using Tukey's HSD Post Hoc. A *p*-value less than 0.05 was considered as statistically significant.

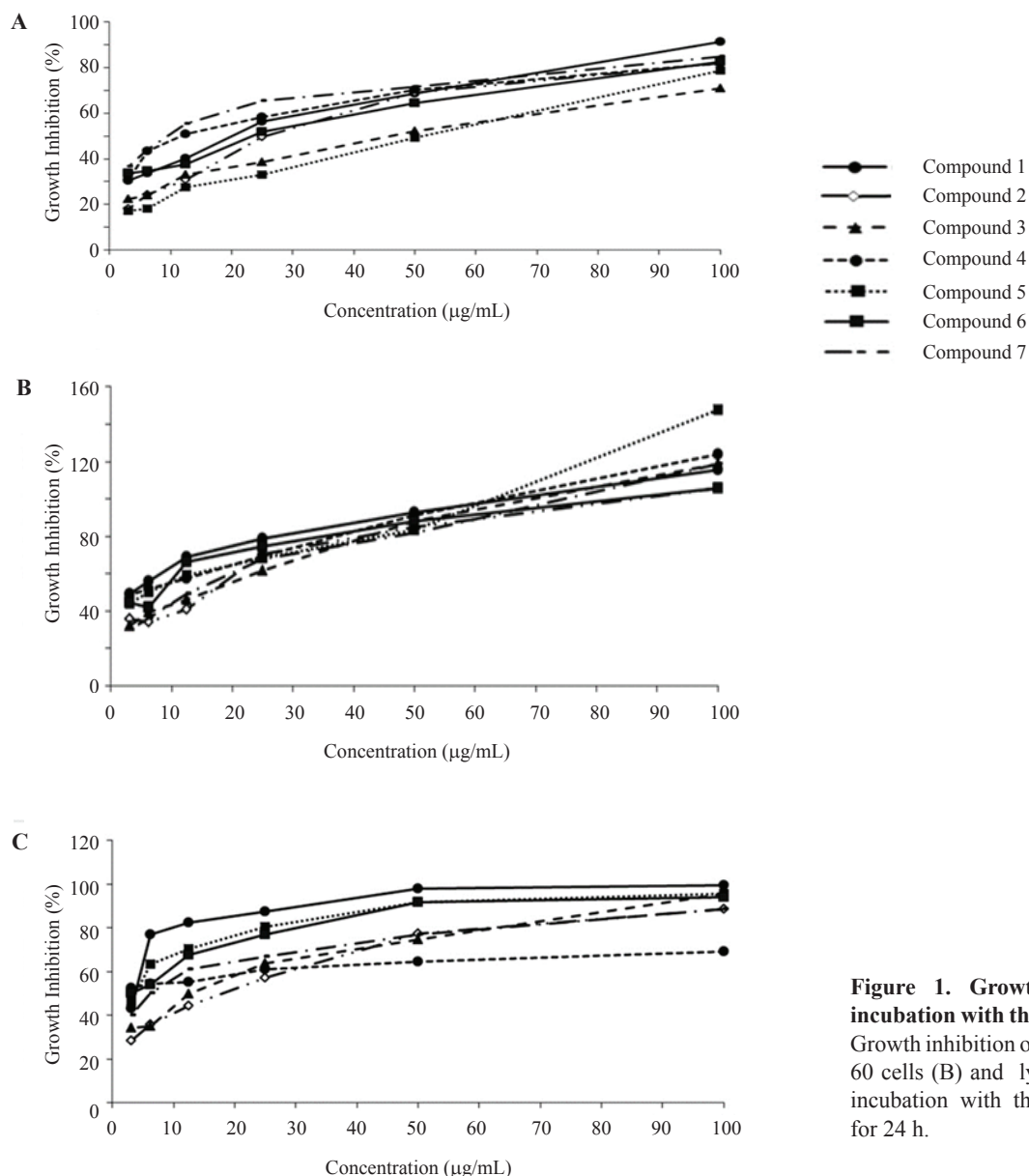
### Results

Growth inhibition of K562, HL-60 cells and lymphocytes after incubation with the tested compounds in various concentrations are presented in Figure 1. The growth inhibition in a dose-dependent manner was observed in all the treated cells by all the tested compounds. At concentration of 100  $\mu$ g/mL, all the tested compounds exhibited more than 70% growth inhibition against the K562 and HL-60 cell lines. Growth inhibition of the lymphocytes after incubation

with the tested compounds at the same concentration were lower than that of the HL-60 cells. It was indicated that the tested compounds more sensitive to the HL-60 cells. The effect of tested compounds toward the K562, HL-60, and lymphocytes cells can be seen in Figure 2, 3 and 4, respectively. It can be seen that mainly the treated cells have lower density than the normal cells.

The cytotoxic activity of the tested compounds against the leukemia cell lines and lymphocytes are presented in Table 2. All seven tested compounds showed moderate cytotoxic activity with IC<sub>50</sub> values ranged from 5.87 to 52.56  $\mu$ g/mL against K562 cell line and from 1.57 to 9.63  $\mu$ g/mL against HL-60 cell line. Among all seven tested compounds, compound 7 showed the most cytotoxic against K562 cell line with the IC<sub>50</sub> value of  $5.87 \pm 0.15$   $\mu$ g/mL, whereas the compound 4 showed the most cytotoxic against HL-60 cell line with the IC<sub>50</sub> value of  $1.57 \pm 0.40$   $\mu$ g/mL.

The SI of the tested compounds are presented in Table 2. Almost all tested compounds showed low selectivity with SI values ranged from 0.05 to 0.96 toward K562 cell line and from 0.45 to 5.26 toward HL-60 cell line.



**Figure 1. Growth inhibition after incubation with the tested compounds.** Growth inhibition of K562 cells (A), HL-60 cells (B) and lymphocytes (C) after incubation with the tested compounds for 24 h.

In comparison, the positive control imatinib showed high selectivity ( $SI > 2$ ) toward both HL-60 and K562 cell line, however accutane showed low selectivity toward K562 cell line. Among all seven tested compounds, the compound 4 showed the highest selectivity toward HL-60 and K562 cell line with SI value of 5.26 and 0.96 respectively, higher than those of imatinib and accutane toward the HL-60 but still lower than imatinib toward the K562 cell line. The compound 5 showed the lowest selectivity toward K562 cell line with SI value of 0.05, whereas the compound 7 showed the lowest selectivity toward HL-60 with SI value of 0.45.

## Discussion

The therapeutic activity of most anticancer drugs in clinical use is limited by their general toxicity to proliferating cells, including some normal cells. Researcher continue to develop novel cytotoxic agent with unique mechanisms of action, but many of that compounds still lack of tumor selectivity and have not been therapeutically useful.<sup>20</sup> Some chalcone derivatives have been synthesized and evaluated for their cytotoxic activity. Various chalcones can inhibit different steps of carcinogenesis from the very early stages, including tumor initiation trough promotion, progression,

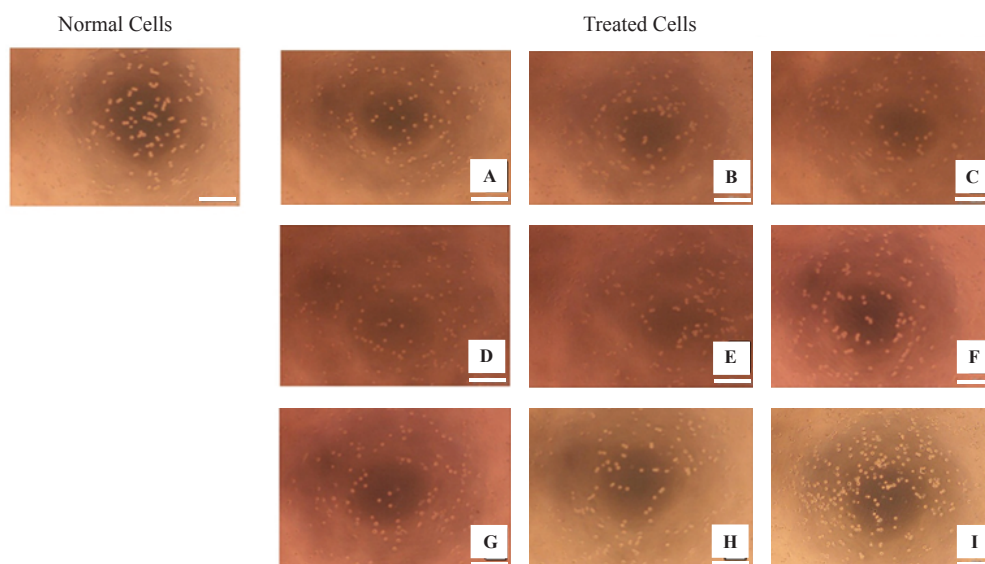
**Table 2. Cytotoxic activity of the tested compounds against the leukemia cell lines (K562 and HL-60) and lymphocytes and their selectivity index.**

Compound number	IC <sub>50</sub> ± SD (µg/mL)			Selectivity Index (SI)	
	K562	HL-60	Lymphocytes	K562	HL-60
1	10.83 ± 0.67 <sup>b</sup>	1.76 ± 0.20 <sup>a</sup>	2.92 ± 1.15 <sup>a</sup>	0.27	1.66
2	21.46 ± 0.30 <sup>c</sup>	7.81 ± 1.21 <sup>b</sup>	10.78 ± 2.09 <sup>ab</sup>	0.5	1.38
3	48.45 ± 5.37 <sup>c</sup>	6.92 ± 1.18 <sup>b</sup>	9.13 ± 4.12 <sup>ab</sup>	0.19	1.32
4	8.65 ± 0.93 <sup>ab</sup>	1.57 ± 0.40 <sup>a</sup>	8.26 ± 2.29 <sup>ab</sup>	0.96	5.26
5	52.56 ± 1.79 <sup>c</sup>	2.49 ± 0.84 <sup>a</sup>	2.46 ± 0.96 <sup>a</sup>	0.05	0.99
6	11.84 ± 1.11 <sup>b</sup>	1.84 ± 0.41 <sup>a</sup>	3.51 ± 0.37 <sup>ab</sup>	0.3	1.91
7	5.87 ± 0.15 <sup>ab</sup>	9.63 ± 2.32 <sup>b</sup>	4.33 ± 0.97 <sup>ab</sup>	0.74	0.45
Imatinib	2.67 ± 0.53 <sup>a</sup>	16.59 ± 0.77 <sup>c</sup>	42.30 ± 17.11 <sup>c</sup>	15.85	2.55
Accutane	39.47 ± 3.14 <sup>d</sup>	7.82 ± 0.92 <sup>b</sup>	20.44 ± 3.72 <sup>b</sup>	0.52	2.61

angiogenesis, and invasion, to the very large states leading to metastasis. Synthesis or chemical modification of chalcones allows rapid generation of large number of novel compounds for anti-cancer.<sup>21</sup> Chalcones contain a common 1,3-diphenyl, propenone template that can be modified through addition of functional groups such as aryls, halogens, hydroxyls, carboxylic groups, benzene, etc to alter the biological potential. The chemical structure of chalcone appears to play critical role in determining their molecular targets.<sup>22</sup> In this study, seven chalcone derivatives compounds cytotoxic activity and selectivity was tested against K562 and HL-60 cells line, then

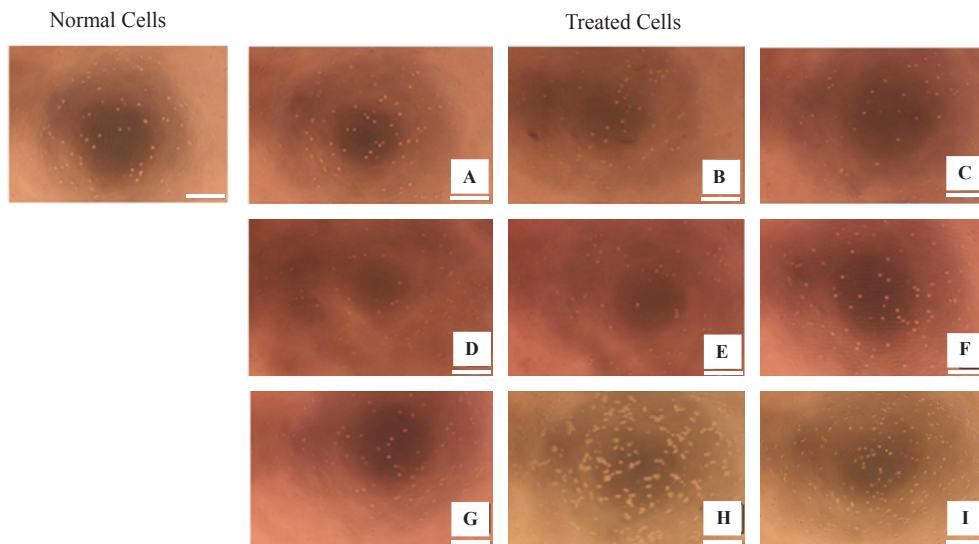
compared with the activity of cancer drugs imatinib and accutane.

The seven derivatives compounds of chalcones exerted cytotoxic activity against K562 and HL-60 cell lines in concentration-dependent manner. Compound 4 and 7 showed highest cytotoxicity against K562 cell lines, comparable with those of imatinib ( $p > 0.05$ ) and better than accutane ( $p < 0.05$ ). Compound 4 also showed highest cytotoxicity against HL-60 cell lines, followed by Compound 1 and 6 with activity were higher than both imatinib and accutane ( $p < 0.05$ ). This study was in accordance with Suwito, *et al.*, study results that Compound



**Figure 2. Morphological appearance of K562 cells with no treatment (normal cells) and cells with treatment at concentration 100 µg/mL.** Treatment cells are compound 1 (A), compound 2 (B), compound 3 (C), compound 4 (D), compound 5 (E), compound 6 (F), compound 7 (G), imatinib (H) and accutane (I). The normal cells relatively has higher density than treated cells. White scale bar: 200 µM.

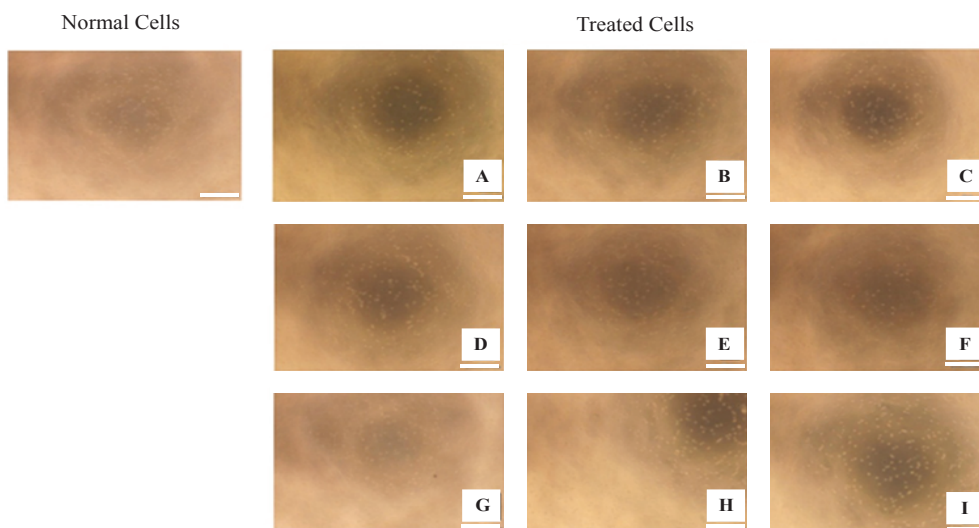




**Figure 3. Morphological appearance of HL-60 cells with no treatment (normal cells) and cells with treatment at concentration 100  $\mu\text{g/mL}$ .** Treatment cells are compound 1 (A), compound 2 (B), compound 3 (C), compound 4 (D), compound 5 (E), compound 6 (F), compound 7 (G), imatinib (H) and accutan (I). The normal cells relatively has higher density than treated cells. White scale bar: 200  $\mu\text{M}$ .

7, 4 and 6 exhibited strongest antiproliferative activity respectively among chalcone derivatives tested against T47D (breast cancer cells). The methoxy-4'-amino chalcone performed better antiproliferative activity compared to methoxy chalcone derivatives and methoxy-4'-bromo chalcone derivatives.<sup>15</sup> These results revealed that amino group attached in 4' position played an important role in the growth inhibition of the cancer cells.

It has been postulated that the presence of 4-amino group on first phenyl ring of the chalcones increases their activity and thus their anti-proliferative activity.<sup>23,24</sup> The  $\text{IC}_{50}$  of almost all seven tested compounds were lower in HL-60 cell lines than K562 cell lines, except for Compound 7. K562 is a kind chronic myeloid leukemia that occurs in blastic myeloid transformation which tends to be more aggressive and treatment resistant than other leukemia.<sup>25</sup> These results



**Figure 4. Morphological appearance of lymphocyte cells with no treatment (normal cells) and cells with treatment at concentration 100  $\mu\text{g/mL}$ .** Treatment cells are compound 1 (1), compound 2 (2), compound 3 (3), compound 4 (4), compound 5 (5), compound 6 (6), compound 7 (7), imatinib (8) and accutan (9). The normal cells relatively has higher density than treated cells. White scale bar: 200  $\mu\text{M}$ .

confirmed that HL-60-human acute promyelocytic leukemia cells are more sensitive than K562 cells.<sup>26</sup>

Our study also confirmed that the number and the position of methoxy group play a role in the chalcone derivatives compound anticancer activity. Two coumarin-containing chalcone compounds were compared, compound with a methoxy group at R3 presented increased levels of inhibition. Activity is due to methoxy group in the architecture.<sup>27</sup> Methoxylated chalcones are structurally similar to combretastatin A-4-5 and colchicine that bind to the tubulin effectively.<sup>28,29</sup> Several other studies also suggested that the number and the position of methoxy substituents on the aromatic rings appeared to be critical for cytotoxicity.<sup>9</sup> Generally, all the seven tested compounds had low selectivity toward K562 cells with all SI values below 2 indicate the compounds had general toxicity to cells.<sup>19</sup> However, Compound 4 showed high selectivity toward HL-60 cells, suggesting it have better potential to be use for acute promyelocytic leukemia.

The mechanism of anti-tumor of chalcones remains to be fully clarified.<sup>30</sup> Some studies reported that chalcone possess the anticancer activity toward HTC116 colon carcinoma cells<sup>31</sup>, leukemia cells (K562<sup>32</sup> and HL-60<sup>8</sup>), HepG2 hepato cellular carcinoma cells<sup>33</sup> prostates cancer<sup>34</sup> and breast cancer cells (MCF-7 and T47D)<sup>35</sup>. In cancer, chalcones disturbs the signal transduction pathway related to cellular proliferation, angiogenesis, metastasis, apoptosis, and the reversal multidrug resistant.<sup>36</sup> In this study the structure of chalcones affected the anticancer and cancer selectivity of chalcones derivatives. Study of molecular mechanisms will be pursued in the further research to confirm the anticancer activity of chalcone derivatives compound 1-7 against leukemia cells.

## Conclusion

Dimethoxy group substitution in the position of carbon 2,3,5 on the second phenyl ring can increase the anticancer activity of 4-amino-chalcones derivatives. The 4-amino-chalcone derivatives with methoxy group in the position of carbon 2,3 on the second phenyl ring possess the highest selectivity toward HL-60 leukemia cancer cell lines among all the tested compounds. The results revealed that the number and position of methoxy groups in chalcone derivatives influenced the anticancer and cancer selectivity of chalcone derivatives.

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