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Anticancer and antimicrobial activity of methoxy amino chalcone derivatives

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ABSTRACT

A series of methoxy amino chalcone derivatives have been synthesized and evaluated for their anticancer activity against breast cancer cells line T47D and their antimicrobial activity against Escherichia coli ATCC 25923, Staphylococcus aureus ATCC 25922, and Candida albicans ATCC 10231. The tested compounds exhibited promising anticancer activity, and compound ***7*** *((E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one) showed the highest anticancer activity (IC_{50} 5.28 µg/mL). Furthermore, in general the prepared compounds also showed good wide spectrum antimicrobial activity, in which their activity equal to sulfamerazine and sulfadiazine used as positive control.*

Keywords: Methoxy amino chalcone derivatives, anticancer, antimicrobial

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INTRODUCTION

Cancer is one of the major world problems due to its increase tendency of cancer cases and deaths. It was reported in year 2007 that 25% of deaths in the developing countries and 15% all of deaths worldwide are caused by cancer [1]. Chemotherapy is one available method to fight cancer. Drugs acting as cytotoxic agent are usually applied in cancer chemotherapy. However, chemotherapy in cancer treatment is usually associated with various side effects [2] and appearance of resistance. In addition, despite of progressive development in cancer chemotherapy, there are still no sufficient cytotoxic agents that act selectively to cancer cells [3]. Another major health problem is the resistance of pathogenic microorganisms to the available antimicrobial agents applied for infectious dissease. Therefore, the need of both new anticancer and antimicrobial compounds is a necessity.

Chalcone (1,3-diphenyl-2-propene-1-one) and its derivatives are known as intermediate in the flavonoids and isoflavonoids biosynthesis. Despite of their widespread in plants, they are found only in small amount through long isolation procedure. However, the interest to chalcones arises in the last decade due to their simple synthesis method and wide spectrum of pharmacological activities, such as antitumor [4], anticancer [5, 6], antimicrobial [7], antimalarial [8], inhibitor of *Pf*FNR-*Pf*Fd [9], anti-inflammatory [10], inhibitor of topoisomerase I [11], and radical scavenger [12]. Furthermore, as demonstrated by several studies, chalcones also act as chemopreventive agents by inhibiting carcinogenesis [13, 14]. Previous study suggested that chalcones are able to induce apoptosis [2]. Based on their wide spectrum of biological activity, chalcones are good candidate could be taken in consideration as promising anticancer and antimicrobial agents.

In continuing of our interest of chalcone in the synthesis and bioactivity point of view, herein we report the design, synthesis, anticancer, antimicrobial activity of methoxy chalcones. The synthesized chalcones are designed attaching 4'-amino group as electron donating, and 4'-brom as electron withdrawing group in ring A. In order to explore the role of this substituent in bioactivity, we synthesized methoxy chalcone attaching no substituent in ring A.

MATERIALS AND METHODS

1. General information

The chemicals used in the research were pro analysis or pro synthesis grade. Melting points were measured with Fisher John melting point apparatus and were used uncorrected. The purity of the synthesized compounds was checked by thin layer chromatography on silica gel GF_{254} plates (E Merck) and the spots were identified by UV-lamp (λ 254 nm). The mass spectra were recorded by HRESI-MS (Waters LCT Premier XE, Waters Corp., Milford, MA, USA) or on ESI-MS spectrometers (LCQ-DECA XP Plus, Thermo-Finnigan, San Diego, CA, USA); NMR spectra were recorded in CDCl3 on a NMR Bruker 400 MHz instrument (400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C, Bruker, Billerica, MA, USA). The IR spectra were recorded on a Spectrum One FT-IR spectrophotometer (Perkin Elmer, Waltham, MA, USA).

2. General procedure of chalcone synthesis

The synthesis of chlacone derivatives was conducted according to the procedure reported by Suwito et al [9] as follows: acetophenone derivative (6 mmol) and benzaldehyde derivative (6 mmol) were mixed and dissolved in 30 mL ethanol in a two neck round bottom flask. To the solution, 6 mL NaOH 40% solution was added drop wise while the temperature was maintained under 10 ^{0}C, and stirred for 1 hour. The stirring was then continued at room temperature for 4 hours. Subsequently, the reaction mixture was poured into ice-water; the precipitated solid was filtered off, and recrystallized from aqueous ethanol.

3. Procedure of anticancer assay

The evaluation of anticancer activity of the prepared compounds was performed using MTT assay [15]. Human breast cancer cells line T47D were seeded in a 96 well plate at density of $1x10^{4}$ cells per well with phenol red-free RPMI 1640 medium (with 10% FBS) and maintained for 24 h. Subsequently, the prepared compounds and doxorubicin as positive control (7 various concentrations) were applied for 24 h. After addition of 0.5% MTT solution as a 1/10 volume of medium in the well, incubation was continued for further 4 h at 37 ^{0}C/5% CO_2. An equal volume of stop solution (0.04 N HCl in isopropanol) to that culture medium was subsequently added to each well and the absorbance at 570 nm (peak) and 630 nm (bottom) was measured after thorough pipetting to disperse the generated blue formazan. It was conducted in triplicate.

Procedure of antimicrobial assay

The antimicrobial activity of the prepared chalcone derivatives was tested against *Escherichia coli* ATCC 25923, *Staphylococcus aureus* ATCC 25922, and *Candida albicans* ATCC 10231. The microbes were grown in different medium (Oxoid), that were EMB (Eosin Methylen Blue) agar for *E coli*, MSA (Manitol Salt Agar) for *S aureus*, and SDA (Sabouraud Dextrose Agar) for *C albicans*. The antimicrobial test was performed using disc diffusion and dilution method [16].

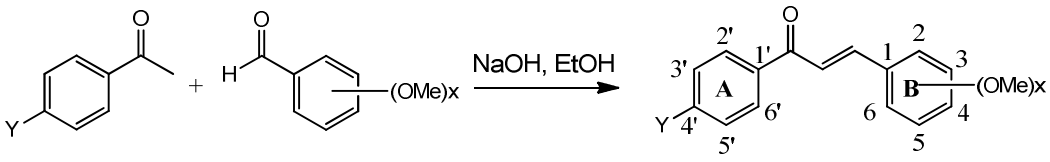
The diffusion method was conducted using Mueller Hinton Agar medium. The tested microbes were suspended in physiologic solution and were measured until optical density (OD) value was 0.5 Mc Farland standard (equivalent with 10^{8} CFU/mL, $\lambda_{625\ nm}$ = 0.08-0.1 for bacterial and $\lambda_{600\ nm}$ = 0.08-0.1 for fungi) (17, Bailey and Scott, 1994). Microbe suspension (1 mL) was grown on a petri disc in a steril MHA medium by pour plate method until the medium solidified. The prepared compounds (25 µL) with different concentration (9 various concentrations) was injected to paper disc (6 mm) and placed on the medium containing tested microbe. It was then incubated for 24 h for bacteria and 48 h for fungi. The inhibition diameter was then measured and it was conducted triplicate.

4. Data analysis

The data were analyzed using program SPSS 21. The data obtained in anticancer assay were analyzed using Probit test, while the average of inhibition diameter of antimicrobial assay were analyzed statistically using Brown-Forshyte and Kruskal-Wallis test and then followed by Games-Howell or Mann-Whitney test at α 0.05.

RESULTS AND DISCUSSION

The present research was focused on the synthesis of methoxy chalcone attaching 4'-amino group, which is known as electron donating and hydrogen bond donor group. This choice was motivated by an article, which reported that 2'-amino chalcone derivatives exhibited potential cytotoxic properties [18]. To explore the role of 4'-amino group of the prepared compounds in anticancer activity, we synthesized 4'-bromo methoxy chalcone derivatives, amino chalcone derivatives attaching no methoxy group in ring B, and methoxy chalcone derivatives attaching no substituens in ring A. The synthesis of the desired chalcones was accomplished by Claisen-Schmidt reaction.



The use of equimolecular of acetophenone and benzaldehyde derivatives in the synthesis of chalcones was to avoid the side products. Under this reaction condition, good to excellent yield was obtained (75-98%), except compound **7** only 42%. The characterization of the synthesized compounds was performed based on spectroscopic evidences, and was already reported from the previous article [9].

Table 1. Structure of the prepared chalcones

Comp	Ring A						Ring B					
	1'	2'	3'	4'	5'	6'	1	2	3	4	5	6
1	--	H	H	NH_2	H	H	--	OCH_3	H	H	H	H
2	--	H	H	NH_2	H	H	--	H	OCH_3	H	H	H
3	--	H	H	NH_2	H	H	--	H	H	OCH_3	H	H
4	--	H	H	NH_2	H	H	--	OCH_3	OCH_3	H	H	H
5	--	H	H	NH_2	H	H	--	OCH_3	H	OCH_3	H	H
6	--	H	H	NH_2	H	H	--	OCH_3	H	H	OCH_3	H
7	--	H	H	NH_2	H	H	--	H	H	H	H	H
8	--	H	H	H	H	H	--	H	H	OCH_3	H	H
9	--	H	H	H	H	H	--	OCH_3	H	OCH_3	H	H
10	--	H	H	Br	H	H	--	H	H	OCH_3	H	H
11	--	H	H	Br	H	H	--	OCH_3	H	OCH_3	H	H

Anticancer activity

The anticancer activity of the prepared compounds was evaluated against breast cancer cells T47D employing MTT cytotoxic assay. The cultivated cancer cells were grown together in the presence of the synthesized chalcones for 24 hours. The amount of the remained viable cells was determined spectrometrically, and doxorubicin was used as positive control. Cells were treated with the synthesized compounds at a concentration range 2.5 – 170 µg/mL. Percentage of viable cells at about 10 µg/mL and concentration required for 50% inhibition (IC_{50}) were determined. The results are shown in Table 2.

Table 2. Results of antiproliferative determination of prepared chalcones against T47D cell lines

Compound	% average of viable cell ± SD at ≈ 10 µg/mL	IC_{50} (µg/mL)
1	61.70 ± 14.88	15.95
2	50.38 ± 10.17	11.96
3	55.91 ± 4.03	11.66
4	46.72 ± 8.29	8.93
5	70.35 ± 8.25	35.40
6	48.14 ± 4.88	8.11
7	33.68 ± 3.42	5.28
8	76.41 ± 5.31	29.32
9	101.68 ± 1.45	48.77
10	107.39 ± 3.59	>> 100
11	99.44 ± 6.49	>> 100
Doxorubicin	---	0.102

Table 3. Inhibition diameter of the prepared compounds against the tested microorganism

Concentration (µg/mL)	Diameter of inhibition ± SD (mm)								
	Comp 1	**Comp 2**	**Comp 3**	**Comp 4**	**Comp 5**	**Comp 6**	**Comp 7**	**SM**	**SD**
***Escherichia coli* ATCC 25923**									
0	7.97± 0.06	7.97± 0.06	7.97 ± 0.06	7.97 ± 0.06	7.97 ± 0.06	7.97 ± 0.06	7.97 ± 0.06	7.97 ± 0.06	7.97 ± 0.06
25	7.97± 0.13	8.04± 0.05	8.08 ± 0.06	8.53 ± 0.16	8.22 ± 0.03	8.27 ± 0.16	8.87 ± 0.28	8.45 ± 0.22	8.33 ± 0.31
50	8.15± 0.14	8.12± 0.09	8.14 ± 0.06	8.80 ± 0.25	8.58 ± 0.06	8.47 ± 0.23	9.42 ± 0.18	8.67 ± 0.23	9.40 ± 0.22
75	8.17± 0.06	8.23± 0.09	8.19 ± 0.06	8.93 ± 0.63	8.82 ± 0.20	8.67 ± 0.42	9.65 ± 0.13	8.93 ± 0.10	9.65 ± 0.28
100	8.23± 0.06	8.37± 0.15	8.23 ± 0.08	8.93 ± 0.38	8.87 ± 0.13	8.85 ± 0.23	9.72 ± 0.10	9.20 ± 0.13	10.58 ± 0.23
200	8.30± 0.10	8.65± 0.22	8.48 ± 0.03	9.45 ± 0.18	9.23 ± 0.19	9.20 ± 0.05	9.80 ± 0.05	9.45 ± 0.10	11.02 ± 0.20
300	9.27± 0.12	9.06± 0.19	8.60 ± 0.57	10.23 ± 0.08	9.43 ± 0.19	9.27 ± 0.37	9.88 ± 0.10	10.08 ± 0.18	12.10 ± 0.18
400	9.42± 0.19	9.45± 0.05	9.07 ± 0.30	10.60 ± 0.17	9.88 ± 0.20	9.50 ± 0.23	10.02 ± 0.03	12.09 ± 0.17	12.73 ± 0.39
500	9.53± 0.15	9.62± 0.08	9.38 ± 0.18	10.68 ± 0.16	10.38 ± 0.10	9.67 ± 0.06	10.25 ± 0.13	13.42 ± 0.08	14.15 ± 0.13
***Staphylococcus aureus* ATCC 25922**									
0	8.16 ± 0.03	8.16 ± 0.03	8.16 ± 0.03	8.16 ± 0.03	8.16 ± 0.03	8.16 ± 0.03	8.16 ± 0.03	8.16 ± 0.03	8.16 ± 0.03
25	8.65 ± 0.16	8.81 ± 0.06	8.81 ± 0.12	9.40 ± 0.02	8.62 ± 0.16	8.12 ± 0.04	8.25 ± 0.10	8.34 ± 0.21	9.09 ± 0.36
50	8.71 ± 0.23	8.98 ± 0.09	9.07 ± 0.26	9.54 ± 0.01	9.12 ± 0.03	8.30 ± 0.35	8.42 ± 0.06	8.42 ± 0.23	9.08 ± 0.08
75	9.08 ± 0.03	9.22 ± 0.04	9.16 ± 0.24	9.74 ± 0.08	9.18 ± 0.06	8.08 ± 0.04	8.63 ± 0.08	8.79 ± 0.41	9.10 ± 0.10
100	9.19 ± 0.04	9.36 ± 0.02	9.36 ± 0.06	9.86 ± 0.01	9.15 ± 0.10	8.24 ± 0.06	8.77 ± 0.08	9.27 ± 0.31	9.22 ± 0.06
200	9.31 ± 0.03	9.41 ± 0.02	9.44 ± 0.07	9.93 ± 0.02	9.33 ± 0.20	8.28 ± 0.16	9.35 ± 0.26	9.55 ± 0.10	9.63 ± 0.10
300	9.42 ± 0.01	9.49 ± 0.08	9.50 ± 0.09	10.11 ± 0.03	9.55 ± 0.17	8.58 ± 0.03	9.37 ± 0.19	10.62 ± 0.10	9.77 ± 0.06
400	9.50 ± 0.03	9.63 ± 0.05	9.52 ± 0.13	10.27 ± 0.03	9.60 ± 0.09	8.78 ± 0.06	9.52 ± 0.03	10.49 ± 0.08	9.65 ± 0.06
500	9.57 ± 0.02	9.78 ± 0.02	9.54 ± 0.19	10.33 ± 0.01	9.75 ± 0.05	8.61 ± 0.20	9.59 ± 0.16	10.66 ± 0.04	9.78 ± 0.03
***Candida albicans* ATCC 10231**									
0	8.13 ± 0.09	8.13 ± 0.09	8.13 ± 0.09	8.13 ± 0.09	8.13 ± 0.09	8.13 ± 0.09	8.13 ± 0.09	8.13 ± 0.09	8.13 ± 0.09
25	7.12 ± 0.04	8.05 ± 0.10	9.75 ± 0.05	8.55 ± 0.09	9.19 ± 0.05	8.14 ± 0.04	8.45 ± 0.22	8.19 ± 0.07	9.06 ± 0.09
50	8.48 ± 0.28	9.43 ± 0.16	10.05 ± 0.14	9.05 ± 0.05	9.56 ± 0.26	8.38 ± 0.09	9.22 ± 0.12	8.38 ± 0.15	9.64 ± 0.12
75	8.92 ± 0.28	9.95 ± 0.09	10.84 ± 0.02	10.05 ± 0.05	10.57 ± 0.23	8.51 ± 0.09	9.38 ± 0.08	8.55 ± 0.05	10.40 ± 0.25
100	9.39 ± 0.05	10.11 ± 0.03	10.64 ± 0.50	10.40 ± 0.20	10.92 ± 0.18	8.83 ± 0.10	9.42 ± 0.21	9.22 ± 0.18	11.16 ± 0.02
200	9.50 ± 0.05	10.13 ± 0.04	11.00 ± 0.15	10.85 ± 0.18	11.15 ± 0.13	9.85 ± 0.07	9.47 ± 0.10	9.62 ± 0.07	11.31 ± 0.05
300	10.03 ± 0.12	10.29 ± 0.12	11.12 ± 0.08	11.21 ± 0.07	11.27 ± 0.08	10.15 ± 0.03	9.50 ± 0.18	9.93 ± 0.24	11.59 ± 0.10
400	10.11± 0.13	10.68 ± 0.08	11.12 ± 0.13	11.28 ± 0.12	11.68 ± 0.05	10.23 ± 0.06	9.58 ± 0.17	10.34 ± 0.04	11.61 ± 0.02
500	11.07 ± 0.03	11.13 ± 0.08	11.17 ± 0.10	11.30 ± 0.15	11.95 ± 0.13	10.29 ± 0.06	9.69 ± 0.02	10.49 ± 0.29	11.66 ± 0.18

Among the tested compounds, methoxy-4'-amino chalcone derivatives (compound **1**-**7**) showed better antiproliferative activity compared to methoxy chalcone derivatives (compound **8**-**9**) and methoxy-4'-bromo chalcone derivatives (compound **10**-**11**). These evidences showed that amino group attached in 4' position played an important role in the growth inhibition of the cancer cells. The role of methoxy group to the antiproliferative activity was studied from the activity of compound **1**-**7**. Compound **1**-**3** is derivative of 4'amino chalcone attaching only one

methoxy group and they showed more or less equally strong. Among compound **4**, **5**, and **6** (dimethoxy derivatives), compound **4** and **6** exhibited equally strong in antiproliferative activity, whereas compound **5** showed low antiproliferative activity. Compound **7**, which attaches no methoxy group, exhibited the strongest antiproliferative activity among the prepared compounds. These evidences showed that methoxy group plays a minor role in the antiproliferative activity.

Antimicrobial activity

The antimicrobial activity of the prepared compounds was determined from the data of minimum inhibition concentration (MIC). Compound **1**-**7** were tested of their antimicrobial activity against *Escherichia coli* ATCC 25923, *Staphylococcus aureus* ATCC 25922, and *Candida albicans* ATCC 10231, whereas sulfadiazine (SD) and sulfamerazine (SM) were used as positive control. The results of the antimicrobial activity assay were tabulated in Table 3.

Without considering the concentration of the tested compounds, statistical analysis showed that in general chemical structure plays an important role to the antimicrobial activity. Against *Escherichia coli* ATCC 25923, compound **4**, and **7** exhibited activity equal strong to sulfamerazine, while the antimicrobial activity of compound **1**, **2**, **3**, **5**, and **6** did not differ significantly. Against *Staphylococcus aureus* ATCC 25922, compound **4** showed the strongest inhibition activity, even compared to sulfamerazine and sulfadiazine. Furthermore, the inhibition activity of compound **1**, **2**, **3**, **5**, and **7** were as strong as sulfadiazine, while compound **6** showed the weakest inhibition activity. Against *Candida albicans* ATCC 1023, the inhibition activity of compound **3**, **4**, and **5** were equal strong as sulfadiazine, while compound **1**, **2**, **6**, and **7** were as strong as sulfamerazine. Based on the results, the prepared compounds exhibited promising antimicrobial agent, especially compound **4** which exhibited the most potential to be applied as the best candidate for wide spectrum antimicrobial agent.

CONCLUSION

The derivatives of methoxy amino chalcone showed promising anticancer activity, especially (*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one (compound **7**). The tested compounds also revealed potential antimicrobial activity, in which (*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (compound **4**) emerged as the most promising compound possessing a wide spectrum antimicrobial activity.

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