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Cytotoxicity of ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) on Helacell line

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ABSTRACT

In our previous study, some amino chalcone derivatives have been synthesized and evaluated their cytotoxicity against breast cancer cell line T47D. Among 11 amino chalcone derivatives, ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) exhibited the most active compound.This study aimed to investigate cytotoxic activity of the ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) against cervical cell line (HeLa). The cytotoxic activitywas determined using the MTT colorimetric assay. Cisplatin was used as positive control. From this MTT method, inhibitory concentration 50% (IC_{50}) values were determined by probit analysis based on the relationship between log concentrations versus the percentage of cells growth inhibition.The results showed that the IC_{50} of ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) and cisplatinwere 22.75 ± 19.13 µg/mL and 14.96±1.08 µg/mL, respectively. In conclusion, the ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) has moderate cytotoxic activity against HeLa cell line based on National Cancer Institute (NCI) criteria.

ABSTRAK

Pada penelitian sebelumnya, beberapa turunan kalkon telah disintesis dan diuji sitotoksiknya melawan sel kanker payudara T47D. Di antara 11 turunan amino kalkon, senyawa ((*E*)-1-(*4-aminophenyl*)-*3-phenylprop-2-en-1-one*)) menunjukkan senyawa yang paling aktif. Penelitian ini bertujuan untuk mengkaji aktivitas sitotoksik senyawa ((*E*)-1-(*4-aminophenyl*)-*3-phenylprop-2-en-1-one*)) melawan sel kanker serviks (HeLa). Aktivitas sitotoksik ditetapkan dengan metode kolorimetri MTT. Cisplatin digunakan sebagai control positif. Dari metode MTT ini, nilai penghambatan 50% (IC_{50}) ditetapkan dengan analisis probit berdasarkan hubungan antara log konsentrasi dengan persen penghambatan pertumbuhan sel. Hasilnya menunjukkan nilai IC_{50} ((*E*)-1-(*4-aminophenyl*)-*3-phenylprop-2-en-1-one*)) dan cisplatin berturut-turut adalah 22,75 ± 19,13 µg/mL and 14,96±1,08 µg/mL. Dapat disimpulkan, senyawa ((*E*)-1-(*4-aminophenyl*)-*3-phenylprop-2-en-1-one*)) mempunyai aktivitas sitotoksik sedang melawan sel HeLa berdasarkan criteria *National Cancer Institute* (NCI).

INTRODUCTION

Cancer is a major public health problem worldwide including in Indonesia. In 2018, it was reported that a total 18 million new cases are diagnosed and 8.97 million death due to cancer in the world.[1] Cervical cancer was the fourth most common cancer in women, ranking after breast cancer (2·1 million cases), colorectal cancer (0·8 million) and lung cancer (0·7 million). In 2018, it was reported approximately 570,000 cases of cervical cancer and 311,000 death from the disease occurred.[2] In Indonesia in 2018, it was estimated that 32,469 women are diagnosed with cervical cancer and 18,279 death from the disease. Cervical cancer ranked as the 2nd most frequent cancer among women in Indonesia.[3,4]

Chemotherapy using cisplatin combined with radiotherapy is the first line treatment for cervical cancer patients.[5] However, patients relapse

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and cisplatin resistance resulting in a poor clinical outcome were reported.[6] Moreover, cisplatin was reported can induce serious side effects such as ototoxic, nephrotoxic, myelotoxic and gastrointestinal toxic may enumerate issues dealing with chemotherapy in cervical cancer patients.[7] Due to the relapse, and anticancer resistance as well as serious side-effect the available of new anticancer is urgently needed. Some anticancer agents such as stanniocalcin 2(STC2), a glycoprotein hormone, have being developed to replace cisplatin resistance.[8]

Chalcones (1,3 -diphenyl -2 -propene -1 -one) are the precursor of flavonoidsynthesis which are abundantly found in many vegetables and fruit. Chalcones have been reported to have various biological activities such as anticancer, antimicrobial, antimalarial, anti-inflammatory, and antioxidant.[9-11] In our previous study, some amino chalcone derivatives have been synthesized and evaluated their cytotoxicity against breast cancer cells T47D. Among 11 amino chalcone derivatives, ((E) -1 -(4 -aminophenyl) -3 -phenylprop -2 -en -1 -one)) exhibited the most active compound.[10] In this study, the cytotoxicity of this compound against cervical cell line (HeLa) was reported.

MATERIALS AND METHODS

Tested compound

The amino chalcone derivatives was synthesized by Suwito *et al.*[9] The structure of this compounds is presented in FIGURE 1.

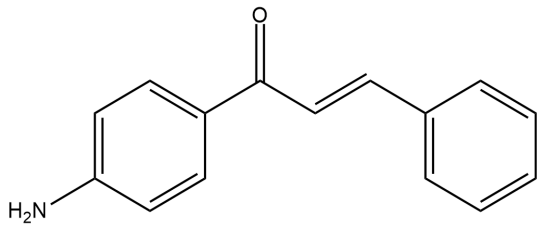


FIGURE 1. Chemical structure of ((E)-1-(4-aminophenyl)-3-phenyl-prop-2-en-1-one)

Cell culture

HeLa cell line was obtained from the collection of the Department of Parasitology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta. The HeLa and Vero cells lines were maintained in RPMI 1640 medium (Sigma) supplemented with 10% fetalbovine serum (FBS) (Sigma-Aldrich, USA), 3% penicillin- streptomycin and 1% fungison. Subcultures were obtained after treatment with 0.05% trypsin (Gibco, Auckland) in phosphate buffer saline. The cells were cultured at 37 ^{o}Cin a humidified 5% CO_2 incubator. The protocol of this study has been approved by the Medical and Health Research Ethic Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta.

MTT assay

Cytotoxic activity of the ((*E*)-1-(-4aminophenyl)-3-phenylprop-2-en-1-one) determined on the cells using the MTT (3 -(4,5 -dimethylthiazol-2-yl)- 2,5 -diphenyltetrazolium bromide) colorimetric assay developed by Mosmann (1983) after modification.[13] One hundred µL of the treated cells culture (HeLa cell) at density of 10^5 cells/well were distributed in 96-wells plates. One hundred µL of culture medium containing the tested comppounds at various concentrations (5 – 160 µg/mL) were added. The medium culture without the tested compounds was used as negative control, whereas cisplatin was used as positive control. The mixture was then incubated at 37 ^{o}C in a 5% CO_2 for 24 h. In the following after incubation, 100 µg/mL of the medium culture was removed and replaced with 100µL solution of 0.5% MTT and then further incubated at 37°C in 5% of CO_2 for 4 h.The reaction was stopped by adding 100 µL of 0.04 HClisopropanol and the mixture was incubated at 37 ^{o}C in a 5% CO_2 for 24 h. The assay was conducted in triplicate in three independents experimental. Absorbance or optical density (OD) for each well was measured in an ELISA microplate readerat λ_{max} of

570 nm. The cell growth inhibition was then determined with the equation.

Data analysis

The absorbance values were directlyproportional to the number of live cells. Theabsorbance values in the presence of ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) were compared with that of negative control to obtain cellsgrowth inhibition. From this MTT method, IC50 values were determined by probit analysis based on the relationship between log concentrations versus the percentage of cellsgrowth inhibition.

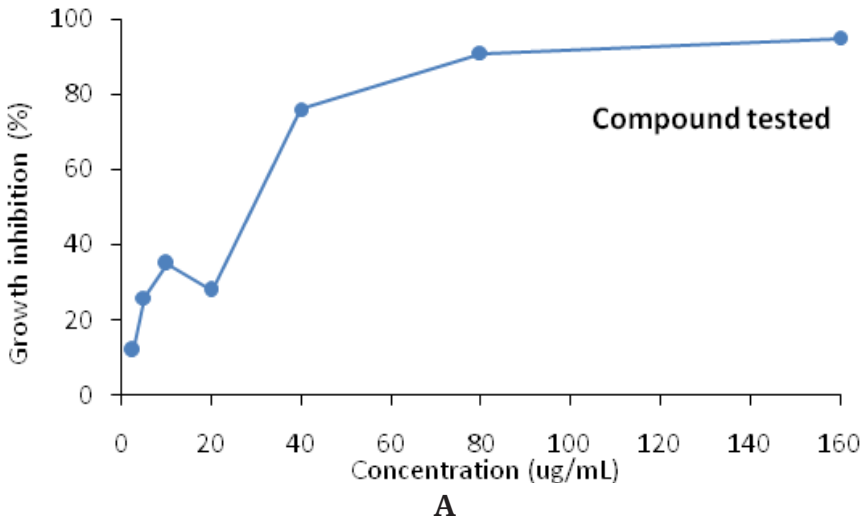
RESULTS

Thegrowth inhibition of HeLa cell line (%) after incubation with ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) or cisplatin for 24 h was presented in TABLE 1 and FIGURE 1. Furthermore, probit analysis showed that the IC_{50} of ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) and cisplatin were 22.75±19.13 µg/mL and 14.96±1.08µg/mL, respectively.

TABLE 1. The growth inhibition of HeLa cell line (%) after incubation with ((E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) or cisplatin.

Compound	Concentration(µg/mL)	Experimental			Mean ± SD	IC_{50} (µg/mL)
		1	2	3		
Compound tested*	160	91.03	99.2	ND	95.1 ± 5.8	22.75 ± 19.13
	80	80.38	97.59	94.13	90.7 ± 9.1	
	40	55.83	91.15	79.37	75.5 ± 18.0	
	20	8.27	47.33	ND	27.8 ± 27.6	
	10	ND	7.75	61.27	34.5 ± 37.8	
	5	ND	12.87	39.62	26.3 ± 18.9	
	2.5	ND	12.29	ND	12.29	
Cisplatin	50	88.7	88.92	88.54	88.7 ± 0.2	14.96 ± 1.08
	25	76.55	72.75	75.43	74.9± 2.0	
	12.5	39.13	38.87	43.52	40.5 ± 2.6	
	6.25	25.48	0	10.39	11.6 ± 13.4	
	3.13	0	0	0	0	
	1.56	0	0	0	0	
	0.78	0	0	0	0	

*((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)); ND: not determined



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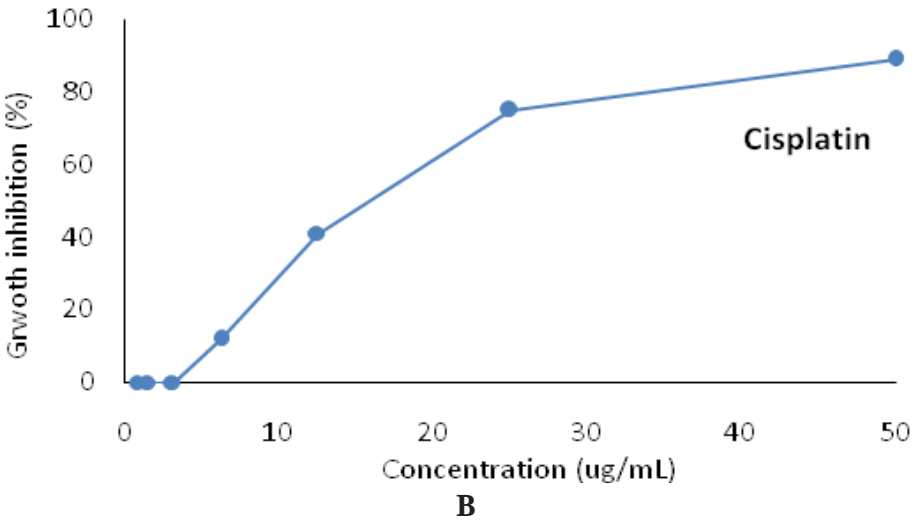

FIGURE 2. Curve of concentration of A) compound tested or ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)); B) cisplatin and the growth inhibition of HeLa cell line (%) after incubation for 24 h.

DISCUSSION

National Cancer Institute (NCI) of US classified the cytotoxic of a compound as high cytotoxic activity if IC_{50}<20 µg/mL, moderate cytotoxic activity if IC_{50} ranged between 21-200 µg/mL, weak cytotoxic activity if IC_{50} ranged between 201-500 µg/mL, and no cytotoxic activity if IC_{50}> 500 µg/mL.[13] Based on this criteria, the ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one))with IC_{50} value of 22.75±19.13 µg/mL was considered to have moderate cytotoxic activity, whereas cisplatin with IC_{50} value of 14.96±1.08µg/mL was considered to have high cytotoxic activity.

A chalcone is a simple chemical scaffold of many naturally occurring compounds and has a widespread distribution in vegetables, fruits, teas, and other plants. Chalcone compounds have a common chemical scaffold of 1,3 - diaryl - 2 - propen - 1 - one, also known as chalconoid, that exists as *trans* and *cis* isomers, with the trans isomer being thermodynamically more stable.[9,14] ((*E*) -1 -(4-aminophenyl) -3-phenylprop -2 -en -1 -one)) is one of amino chalcone derivatives which have been synthesized and evaluated its cytotoxic activity against breast cancer cell line (T47D) in the previous study. ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) was the most active against T47D among the derivatives tested with an IC_{50} of 5.28 µg/mL.[10]

Chalcones possess an interesting spectrum of pharmacological activities, including anti oxidative, anti bacterial, anti -inflammatory, cytotoxic, immunosuppressive and anticancer, potential. Numerous chalcones appear to show cytotoxic activity against various cancer cells lines, suggesting that chalcones may be considered as potential anticancer drugs. As anticancer, chalcones have been shown to interfere with each step of carcinogenesis, including initiation, promotion and progression.[15] Furthermore, tremendous effort has been conducted to investigate the mechanisms of action of the chalcones and their derivatives. In different screening assays, chalcones have been able to target multiple cellular molecules, suchas MDM2/p53, tubulin, proteasome, NF-kappaB, TRIAL/death receptors and mitochondria mediated apoptotic pathways, cell cycle, STAT3, AP-1, NRF2, AR, ER, PPAR-γ, β- catenin / Wnt and others.[16]

From this study, ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) has moderate cytotoxic activity. Further study will be conducted to

modify this compound in order to obtain the new derivatives which more active. Evaluation of cytotoxicity of this compound against normal cell line will also conducted in order to obtain its index selectivity.

CONCLUSION

In conclusion, the ((*E*)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one))exhibits moderate cytotoxic activity against HeLa cell line. Further study, will be conducted to modify and investigate index selectivity of this compound.

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