Synthesis, Antioxidant and Toxicity Activity of Compounds (E)-1-(3-bromophenyl)-3-p tolylprop-2-en-1-on

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Abstract: Halogen substituted analog compound chalcone (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on was synthesized from 4-metylbenzaldehyde as aldehydes with 3- bromoacetophenone, as ketones by using aldol condensation reaction. The compound resulted rendement with value of 62,38% and characterized by using UV, IR, MS, and 1HNMR. Test of antioxidant activity using DPPH method showed that those compounds have low potency as antioxidant agent LC_{50} with value 571, 7903 ppm. Toxicity tests using Brine Shrimp Lethality Test (BSLT) showed that those compounds have a potency as anticancer agent with LC_{50} value 7,94 µg/mL.

1 INTRODUCTION

Chalcones (α, β-unsaturated aromatic ketones) are medicinally important compounds (Zhuang, 2017). Chalcones have been targeted by several researchers in recent years due to their wide biological potentials (Espinoza, 2016). Various natural and synthetic chalcones have been shown to display biological properties to act as potential hits for anticancer (Park et al, 2018; Michelini, 2018), Antitumor (Fouad, 2018), Antioxidant (Jawad, 2018).

Antioxidants are compounds that have the ability to ward off free radicals that can cause various dangerous diseases such as cancer, cardiovascular diseases and degenerative diseases (Barhe, 2014). Antioxidant testing in this study used the DPPH (1,1-diphenyl-2-picrylhydrazyl) method by spectrophotometry, because this method has the advantages of being simple, easy, fast, sensitive and requires only a few samples in testing. The parameters used in this method are IC_{50}, which is the sample concentration needed to capture DPPH radicals by 50% (Polo, 2019).

Cancer is a large group of heterogeneous diseases characterized by abnormal division and spread of cells (Mansoori et al, 2017). 2,2-dimethyl-2,3-dihydro-4(1H)-quinolinone were screened against the NCI-N87 and DLD-1 cancer cell lines, with most compounds showing low micromolar cytotoxic activity (Jean, 2018).

Chalcone compounds can be synthesized through Claisen-Schmidt condensation from an aldehyde and aromatic ketone with an acidic or basic catalyst. The basic catalysts commonly used are NaOH (Suwinto, 2014) and KOH (Brahmana, 2015; Riaz, 2019). Whereas commonly used acid catalysts are HCl (Wang, 2019), H_2SO_4 (Dong, 2018) and $HClO_4$-SiO_2 (Siddiqui, 2015).

Chalcon synthesis in this study used the Microwave Assisted Organic Synthesis (MAOS) method. Microwave-induced organic reaction enhancement (MORE) chemistry is gaining popularity as a non-conventional technique for rapid organic synthesis. Important features of this technique are easy access to very high temperature, good control over energy input in a reaction, higher

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yields and rapid synthesis of organic compounds (Ahmad, 2016). Microwave-assisted organic synthesis (MAOS) has been risen as new strategy in the syntheses of dyes with luminescence properties. Scientists have turned from conventional heating to microwave assisted chemistry as an astounding and powerful strategy for their researches in modern organic synthesis. The technique offers a lot of advantages as it is simple, rapid, economic, and efficient. Dyes are effective molecules have pivotal effects in various fields (Elgemeie, 2018).

However, if viewed from its biogenetic origin, halogen-substituted chalcone is not possible in nature. Therefore, to obtain halogen substituted chalcons, synthesis is carried out. Based on the description above, it can be seen that chalcone has been widely used for various medical purposes. The chalcon compounds to be synthesized are chalcone substituted with halogen (E) -1- (3-bromophenyl) -3-p-tolylprop-2-en-1-on. The compounds were characterized by UV, IR, MS, and ^{1}H-NMR analysis. The compounds were tested for their cytotoxic activity and antioxidant activities by standard methods.

2 MATERIALS AND METHODS

2.1 General Information

The chemical used in the research were pro analysis grade. Melting points were measured with a Fisher Johns melting point apparatus (SMP 11-Stuart®). The purity of synthesized compounds was checked by thin layer chromatography on silica gel GF254 plates, the eluent was mixture of n-hexane/ethyl acetate in 9:1 ratio and the spot were identified by UV (Camag®) (254 nm) . The mass spectra was recorded by MS Water LCT Premier XE spectrometers, NMR spectra was recorded in CDCl3 on a NMR (AGILENT 500 MHz). The IR spectra was recorded on a spectrum FTIR (Shimadzu, IR Prestige-21) spectrophotometer. The UV-Vis spectra was measured on UV-Vis (Genesys 10S®) spectrophotometer. Chalcone synthesis used oven microwave Samsung ME 109 F.

2.2 General Procedure for Chalcone Synthesis

A mixture of 3- bromoacetophenone (5 mmol) was dissolved in ethanol (5 mL), then KOH 6 N (2 mL) was added dropwise. The mixture was stirred for 5 minutes and 4-metylbenzaldehyde (5 mmol) added to the mixture. The mixture is irradiated using microwave for 2-5 minutes, with an interval of 30 seconds. After that, the mixture is left for 20 hours to maximize the results of the reaction (sediment) obtained. A total of 15 mL of cold distilled water was added to the mixture and the pH of the mixture was neutralized with HCl. The precipitate formed is then filtered with a Buchner funnel, washed with cold n-hexane, and vacuumed to dry. Stages of reaction were observed with TLC. The product obtained was tested for its purity by TLC test and melting point. The pure product obtained was then determined by UV, IR, MS and ^{1}H-NMR spectroscopy.

2.3 Antioxidant Activity

DPPH (1,1–diphenyl-2-picrylhydrazyl) radical scavenging activity was measured by the method of Lamaison et al. The reaction mixture contained 1.5×10^{-7} M methanolic solution of DPPH and various concentrations of the test substances and was kept in the dark for 50 min. Optical density (OD) of samples was measured at 517 nm against a blank, and IC50 values were calculated (using linear regression analysis) by plotting a graph, taking concentration on the X-axis and percentage inhibition on the Y-axis, at 50% of the percentage inhibition the line was drawn from Y-axis and aligned with the concentration on X-axis then the IC_{50} values were obtained.

2.4 Brine Shrimp Lethality Bioassay (BSLT)

Brine shrimps (Artemia salina) was hatched using brine shrimp eggs in a conical flask (1 L), filled with sterile artificial sea water under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from the brighter portion of the chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 5 mL of brine solution. In each experiment, test substances whose activities are to be checked were added to the vial according to their concentrations and maintained at room temperature for 24 h under light and the surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations (10, 100 and 1.000 µg/mL) of the test substances in a set of three tubes per dose. Replicas should be maintained to get accurate results.

3 RESULTS AND DISCUSSIONS

(E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on (1) : yellow crystals (0.9358 g ; 62.38%), m.p 131-1320C, Rf = 0,79 (n-hexane/ethyl acetate: 9:1); Mass spectrum (HR-MS) m/z: 300,0152 with formula C16H13Obr; IR (KBr, cm-1): 792 (C-Br), 1512 (C = C of benzene), 1604 (C = O of ketone), 2362 (C-Br), 2960 (-CH3), 3061 (CH from benzene) and 3468 (Overtone from C = O); 1H-NMR (CDCl3, 500 MHz) δH 8.13 ppm (s; 1H); 7.93 ppm (d: 7.5; 1H); 7.81 ppm (d: 15.5; 1H); 7.70 ppm (dd: 8; 1; 1H); 7.55 ppm (d: 8; 1H); 7.42 ppm (d: 15.5; 1H); 7.38 ppm (t: 8; 1H); 7.24 ppm (d: 7.5; 1H); 2.40 ppm (s; 3H).

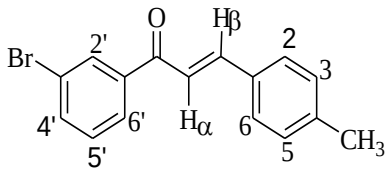

Figure 1: Chalcon Structure.

Compound is synthesized via an aldol condensation reaction, where a new carbon-carbon bond is formed between α carbon atoms from one carbonyl and another carbonyl carbon atom. The acidity of the hydrogen atom α from the carbonyl compound allows the carbonyl compound to react with the others to produce a combined product of both. This reaction is catalyzed by a base (KOH).

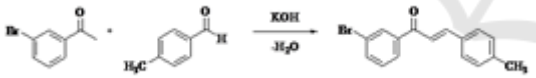

Figure 2: Synthesis reaction of chalcone compounds (E)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on.

DPPH is a stable free radical and is used to evaluate the reduction of free radicals that have a principle that DPPH will be reduced by the donation process of hydrogen or electron so that the color will change from violet to yellow with changes in color intensity proportional to the number of electron donations followed by a decrease in DPPH absorbance (Dris and Jain, 2004). Where, the greater the decrease in absorbance of DPPH, the stronger the antioxidant activity.

The antioxidant value of the extract was determined based on IC_{50} value, namely the concentration of chalcone which caused a reduction of DPPH activity by 50%. The smaller the IC_{50} value, the more active the sample is in capturing DPPH radicals or better antioxidant activity (Morales, 2013).

The percentage of inhibition was determined by comparing the DPPH absorbance purely with the absorbance of DPPH plus chalcone at a wavelength of 516.5 nm. The results showed that DPPH inhibition by chalcons was directly proportional to the concentration of chalcone, which meant that the greater the concentration of chalcone, the higher the inhibition percentage. Chalcone compounds work as antioxidants by breaking radical chain reactions and donating hydrogen atoms to produce more stable free radicals (Nimse and Pal, 2015). The antioxidant ability of chalcone has an IC_{50} value of 571, 7903 ppm and is classified as a weak antioxidant (Jacoeb et al, 2011).

Toxicity activity tests were carried out on *A.salina* larvae using the Brine Shrimp Lethality Test (BSLT). The selection of this method as an initial screening in an effort to search for anticancer compounds because of the low cost of the experiment, the process is fast and simple. In addition, these larvae have several advantages including easy to obtain, easy to breed and can live in a high range of salinity. The larvae are obtained by hatching eggs for 48 hours. Hatched larvae will swim to a bright place. This will make it easier for the separation and retrieval of these animals that have become larvae.

Each of the compounds to be tested was made in concentrations of 1000, 100 and 10 µg/mL in seawater for 24 hours of testing. The difference in concentration is intended to determine the level of activity of each compound against the death of these larvae. Making test solutions using ethyl acetate solvents because the analogue compound chalcone tested was dissolved in ethyl acetate. The solvent is left until it evaporates perfectly so as not to interfere with the toxicity tests carried out. Before adding sea water, dimethyl sulfoxide (DMSO) is added to help dissolve the test compound in seawater so that the compounds can be distributed evenly. The amount of DMSO added is 50 µL, because if more than 50 µL can cause death in the larvae. In this test DMSO was used as a control, which was not too toxic as the reason for choosing DMSO to help dissolve compounds in seawater.

Halogen-substituted chalcone compounds have significant cytotoxic activity with an LC_{50} value of 7.94 µg /mL. The analogue compounds of chalcone tested showed toxic effects on the death of A.salina larvae and showed potential toxicity. Because a compound is said to be active if the $LC_{50} \leq 250$

µg/mL and a maximum of 500 µg/mL (Meyer, 1982).

The biological activity of chalcone compounds in this study, may be influenced by the presence of α, β-unsaturated carbonyl groups and substituents bound to the aromatic ring contained in the compound. (Suvitha, 2012) argue that the possibility of chalcon compounds can induce cell death by interfering with mitochondrial function as cell respiration. Cell respiration is the oxidation process of food molecules, for example glucose to CO_2 and H_2O which form energy in the form of ATP (Adenosine Tripospat) which is useful in supporting cell activity that requires energy. If mitochondrial damage occurs in the cell, it will cause interference with mitochondrial function in ATP synthesis so that the cell will die. Cell death will later cause the death of the larvae themselves.

4 CONCLUSIONS

In conclusion, we have designed and synthesized in good Halogen substituted analog compound chalcone (*E*)-1-(3-bromophenyl)-3-p-tolylprop-2-en-1-on using aldol condensation reaction reactions with yield 62,38%. Test of antioxidant activity using DPPH method showed that those compounds have low potency as antioxidant agent LC_{50} with value 571, 7903 ppm. Toxicity tests using Brine Shrimp Lethality Test (BSLT) showed that those compounds have a potency as anticancer agent with LC_{50} value 7,94 µg/mL.

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