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Synthesis and Toxicity Assessments Some *para*-methoxy Chalcones Derivatives

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Abstract

Chalcones is a very interesting compounds because it is known to have various of biological activities such as antimicrobial, antifungal, anticancer, antimalarial, antioxidant, antitumor, anti-inflammatory and antidepressant. Moreover, natural and synthetic compounds of chalcones have roles as precursors for other compounds. Therefore, many chalcones become model structures of target compounds by researcher. In this research, methoxy chalcones derivatives have been synthesized using stirrer method and using base catalyst NaOH. The synthesized results obtained are (*E*)-3-(4-isoprophylphenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one (1), (*E*)-1-(4'-methoxyphenyl)-3-*p*-tolylprop-2-en-1-one (2) and (*E*)-3-(3-bromophenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one (3). The purity of all compounds have been tested using TLC, melting point test, analytical HPLC. Then they are characterized using UV, FTIR, ^{1}H-NMR and MS spectroscopy. The toxicity assessments of the novel compounds were done by *Brine Shrimp Lethality Test* (BSLT) method. The all compounds showed very good activity with LC_{50} value<200 µg/mL.

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Keywords: chalcones; toxicity assessments; *Brine Shrimp Lethality Test* (BSLT)

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Nomenclature

µL	microliter	t_R	time retention
µg	microgram	LC_{50}	lethal concentration 50

1. Introduction

Chalcones is one of secondary metabolite compounds, which are of interest due to their various biological activities as antimicrobial[1], antifungal[2], anticancer[3], antimalarial[4], antioxidant[5], antitumor[6], anti-inflammatory[7] and antidepressant[8]. Chalcones is also an important precursor for biosynthesis process of flavonoids[9] and isoflavonoids[10] and also several heterocyclic compounds such as benzodiazepine[11], pirazoline[12], flavone[13] and aurone[14]. Chalcones have two aromatic rings and connected by three carbon α,β unsaturated in carbonyl compounds system[15](Fig. 1).

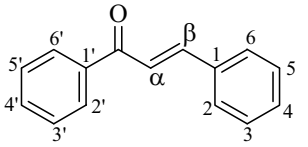


Fig. 1. Structure of chalcones

Chalcones synthesis is normally by condensation of an aromatic aldehydes and ketones in acidic or basic conditions. This method is known as aldol condensation and most often using Claisen-Schmidt condensation[16]. This reaction could be catalyzed by both acid and alkaline. The catalyst that often used for that reaction was HCl[17], $SOCl_2$[18], NaOH[19] and KOH[20].

Among activities of chalcones, cytotoxic activity is typically interesting because it potentially can be use as anticancer drugs. Chalcones methoxy derivatives known to have good cytotoxic activities[21,22]. Therefore, we have synthesized chalcones containing methoxy groups in *para* position. These compounds were tested toxicity assessments with *Brine Shrimp Lethality Test* (BSLT) method. Finally toxicity of these *para*-methoxy chalcones derivatives were experimentally and theoretically evaluated as anticancer compounds.

2. Material and methods

The main materials are 4-methoxy-acetophenone (Merck), 4-isoprophyl-benzaldehyde (Merck), 4-methyl-benzaldehyde (Merck), 3-bromo-benzaldehyde (Merck), NaOH (Merck), HCl (Merck) and larva of *A. Salina*. Melting point of all that compounds were determined by Fisher Johns point apparatus. The chromatogram HPLC was performed using Shimadzu LCsolution instrument. The UV-Vis spectra were performed using UV-Vis spectrophotometer (Genesys 10S UV-Vis v4.002 2L9N175013). The IR spectra were recorded on FTIR, Shimadzu, IR Prestige-21. Mass spectra were measured by mass spectrometer using water LCT Premier XE postive mode. ^{1}H-NMR spectra were measured by NMR spectrometer using JEOL Type ECA 500 MHz.

2.1 Synthesis of chalcones

4-metoxyacetophenone (5mmol) and ethanol (10 mL) were stirred in round-bottom flask and catalyzed by NaOH (5%, 5 mL). Then, benzaldehyde derivatives (5 mmol) was added and the reaction mixture was stirred for 4 hours in room temperature and monitored by TLC. It was kept for 18-24 hours. Then cold aquadest (10 mL) was added on it and the pH was neutralized with HCl. The solid layer was separated and washed with cold *n*-hexane. The purity of product then tested using TLC, melting point test and analytical HPLC.

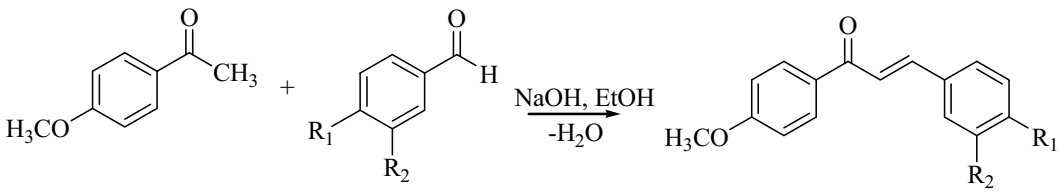
2.2 Toxicity assessments

Toxicity assessments were performed by *Brine Shrimp Lethality Test* (BSLT) method[23]. Each synthesized compounds were dissolved in methanol to make 10,000 µg/mL main solution. The main solution was diluted to make 10, 100 and 1000 µg/mL solution. The methanol solvent was vaporized in room temperature to remove its solvent. The compounds were then redissolved in 50 µL of DMSO. Sea water and 10 larva of *A. salina* were added into the solution. After 24 hours, the number of the death larva was counted and calculated LC_{50} value with curve method using probit analysis table.

3. Results and discussion

3.1 Chemistry

Chalcones derivatives were produced by reacting 4-methoxy acetophenone with 3 aromatic aldehyde derivatives (4-isoprophylbenzaldehyde, 4-methylbenzaldehyde and 3-bromobenzaldehyde) using NaOH as catalyst in ethanol (Fig. 2). Ethanol was used as solvent due to its low toxicity and ability to absorb water which was the side product of this reaction.



(1) $R_1 = C_3H_7$ $R_2 = H$
(2) $R_1 = CH_3$ $R_2 = H$
(3) $R_1 = H$ $R_2 = Br$

Fig. 2. General scheme for synthesis of chalcones

The products of synthesis were (*E*)-3-(4-isoprophylphenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one (1), (*E*)-1-(4-methylphenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one (2) and (*E*)-3-(3-bromophenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one (3) (Table 1). Chalcones (1) with lowest yield is probably due to the isoprophyl group which is an electron donating group causing the aldehyde became less positive, as the result the carbonyl group became less reactive to react with enolate ion from the aromatic ketone therefore reduce its yield. Chalcones derivatives that were produced then were tested with melting point test, TLC and HPLC. Melting point test produced the melting point within 2^0C range. TLC analysis using ethyl acetate : *n*-hexane (1:7) as eluent produced 1 spot. HPLC analysis at λ=210 nm for each chalcones produce a single peak at t_R=16.2 min (1), t_R=14.5 min (2) and t_R=15.2 min (3). This result proved that the product was pure.

Table 1. Results synthesis of chalcones derivatives

Chalcones	Molecular Formula	Mass	Yield	Shape	Colour	Melting Point (^{0}C)
1	$C_{19}H_{20}O_2$	280,15	57 %	Needle like crystal	Opaque	75-77
2	$C_{17}H_{16}O_2$	252,15	99 %	Powder	White	129-131
3	$C_{16}H_{13}O_2Br$	316,01	99 %	Powder	White	126-128

UV spectra showed λ_{max} in the range of 201-203 nm which are signature of benzene group. Another peak at 280-290 nm is due to carbonyl group signature peak.

IR (KBr) spectra produced peaks in the range of 3013-3022, 1509-1511, and 1652-1659 cm^{-1} which are the signature absorption of aromatic C-H, conjugated C=C and carbonyl group (C=O). Chalcones (1), $\nu(cm^{-1})$: 3013(C-H aromatic), 1509(C=C), 1655(C=O), 1265(Ar-OCH_3), 2879(Ar-CH_3). Chalcones (2), $\nu(cm^{-1})$: 3019(C-H aromatic), 1510(C=C), 1652(C=O), 1248(Ar-OCH_3), 2844(Ar-CH_3). Chalcones (3), $\nu(cm^{-1})$: 3022(C-H aromatic), 1511(C=C), 1659(C=O), 1258(Ar-OCH_3), 2843(Ar-CH_3), (C-Br).

^{1}H-NMR ($CDCl_3$) spectra produced distinctive chemical shifts at δ = 7.51-7.53 ppm (d, 1H, Hα, J = 15.6 Hz) and δ = 7.71-7.79 ppm (d, 1H, H_β, J = 15.6 Hz) which are probably due to trans-configuration on the double bond. Chalcones (1), δ (ppm): 8,04(d, 2H, J = 9,1 Hz), 7,79(d, 1H_β, J = 15,6 Hz), 7,58(d, 2H, J = 7,8 Hz), 7,51(d, 1Hα, J = 15,6 Hz), 7,28(d, 2H, J = 7,8 Hz), 6,98(d, 2H, J = 9,1 Hz), 3,89(s, 3H), 2,94(m, 1H) and 1,27 (d, 6H, J = 7,2 Hz). Chalcones (2), δ (ppm): 8,04(dd, J_1 = 1,9 Hz, J_2 = 6,5 Hz, 2H), 7,79(d, 1H_β, J = 15,6 Hz), 7,54(d, 2H, J = 8,5 Hz), 7,51(d, 1Hα, J = 15,6 Hz), 7,22 (d, 2H, J = 8,5 Hz), 6,98(dd, 2H, J_1 = 1,9 Hz, J_2 = 7,1 Hz), 3,89(s, 3H) and 2,39(s, 3H). Chalcones (3), δ (ppm): 8,04(dd, 2H, J_1 = 1,9 Hz, J_2 = 7,2 Hz), 7,79(s, 1H), 7,71(d, 1H_β, J = 15,6 Hz), 7,53 (d, 1Hα, J = 15,6 Hz), 7,52 (d, 2H, J = 7,1 Hz), 7,29(t, 1H), 6,99(dd, 2H, J_1 = 1,9 Hz, J_2 = 6,5 Hz) and 3,89(s, 3H).

MS (*m/z*) spectra produced peaks that consistent with the predicted molecule. Chalcones (1), (2) and (3) predicted peak of $(M+H)^+$ 281.1542, 253.1229 and 317.0117 while the experimental peak was 281.1535, 253.1226 and 317.0185 consequently. Spectra of chalcones (3) also shown a peak near $(M+H)^+$ with the same height which is caused by the existence of ^{79}Br and ^{81}Br isotops. The difference between theoretical and experimental peak at MS spectrum is insignificant, it proves that the elucidated compound have the same structure as the expected compound.

3.2 Toxicity assessments with BSLT method

The result of toxicity assessments were positive. LC_{50} value of chalcones (1), (2) and (3) consequently were 12.68, 79.62 and 13.06 µg/mL, therefore the toxicity level of those 3 compound were (1) > (3) > (2). This results show chalcones derivatives were potential candidate as anticancer compounds due to its LC_{50} value that were lower than 200 µg/mL[24]. However, further cytotoxic assessments are needed to prove that those compounds can be used as anticancer.

Conclusion

Chalcones that have been succesfully synthesized are (*E*)-3-(4-isoprophylphenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one, (*E*)-1-(4-methylphenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one and (*E*)-3-(3-bromophenyl)-1-(4'-methoxyphenyl)prop-2-en-1-one using stirring method and NaOH as the catalyst with 57-99% yield. Toxicity assessments results show that all of them are potential candidate as anticancer compounds (LC_{50}<200 µg/L).

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