Synthesis and Brine Shrimp Bioassay of Chalcone and Its Two Methoxy Derivatives

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Research Article

Synthesis and Brine Shrimp Bioassay of Chalcone and Its Two Methoxy Derivatives

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ABSTRACT

Chalcone and its two methoxy derivatives have been synthesized by a simple Claisen-Schmidt condensation in presence of NaOH 60%. The reactions were carried out at room temperature for about 15 -105 minutes and provided the desired compounds in about 73-96% yields. The structure of the compounds was confirmed by ^{1}H-NMR, ^{13}C-NMR, IR, MS and UV-vis spectroscopic methods. Furthermore, all the synthesized compounds were examined their cytotoxic action by BST method (brine shrimp lethality test) and expressed as toxic compound.

Keywords: chalcones, aldol condensation, synthesis, brine shrimp test, cytotoxic.

INTRODUCTION

Chalcones are compounds which naturally occurrence in a great number of edible plants.[1,23] They are belonging to flavonoid family[26] and also considered to be a main precursor in biosynthesis of various biologically essential heterocycles such as benzothiazepines, pyrazolines, pyrimidines, flavonoids, isoflavonoids and and flavones[1,23,26]. Chalcones are 1,3-diphenyl-2-propene-1-ones, wherein two benzene rings are connected by highly electrophillic three carbon α,β-unsaturated carbonyl structure[1,23,26]. Furthermore, chalcones are important intermediates in many addition reactions of nucleophiles owing to inductive polarization of carbonyl group at the β-position[27]. These facts clarify the significant interest of scientist in this particular group of compounds.

Chalcone and its derivatives have also attracted vast attention due to numerous pharmacological properties[2,23,26]. The compounds with chalcone's skeleton have been reported to possess a broad spectrum of biological activities such as anti-inflammatory activity[1,23,27], chemopreventive activity[1], anticancer activity[27], antiproliferative activity[1], antimalarial activity[1,23,26,27], antiparasitary activity[2] and anti HIV activity[1]. Some chalcones display antimicrobial[2,23,25,26,27], antifungal[1,27], insecticidal[1], anti-ulcerative[27] and anti hyperglycemic properties[23]. Some of their derivatives exhibit analgesic activity[1], antipyretic activity[27], antioxidant[2,4,23,26,31], antiviral activity[1], anti tumor activity[2,27] and cytotoxic activity[1,2,23,27].

There are several methods for the syntheses of chalcones have been investigated[6,23,26]. The major synthetic schemes are including Claisen-Schmidt condensation[1,23,24,26,27], Suzuki coupling reaction[10,27], Wittig reaction[24], Friedel-Craft acylation[24,27], photo-Fries rearrangement[27], Carbonylative Heck reaction[9] and also unconventional method via microwave irradiation[2,3,7,23,27,30]. On the Suzuki coupling reaction, benzoyl chloride was reacted with phenylvinylboronic acid using anhydrous toluene and catalyzed by tetrakis(triphenylphosphine)palladium(0) and cesium carbonate as base, gave 3',4',4-trimetoxychalcone[10,27]. In Friedel-Craft acylation, 2',4',4'-trihidroxy-3',5'-dimethylchalcone was synthesized by direct acylation of a phenol derivatives with cynnamoyl chloride[27]. In photo-Fries rearrangement, phenyl cinnamate undergoes rearrangement and provided two chalcone molecules, *ortho* and *para*-hydroxy chalcones[27]. In carbonylative Heck reaction, chalcone was produced by the reaction of aryl halide and styrene that added by carbon monooxide and catalyzed by palladium and a phosphine-amine ligand, *N*-heterocyclic carbene[9].

The Claisen-Schmidt condensation is a classical method to synthesize chalcones which employs cross aldol condensation of appropriate aldehyde and ketone by base catalyzed (such as NaOH, KOH, $Ba(OH)_2$, Na_2CO_3, hydrotalcites, etc)[1,27] or acid catalyzed (such as HCl, BF_3, B_2O_3, *p*-toluenesulfonic acid, $SOCl_2$, etc)[27] and then followed by dehydration[23]. Nevertheless, many of these methods require prolonged reaction times, gave poor yields, low selectivity and also suffered from harsh reaction condition for instance toxic reagent, strong acidic and strong basic condition. From many literatures, Claisen-Schmidt condensation still occupies leading positions for synthesizing chalcone, since that method is very simple, inexpensive and easy to conduct[26,27].

In vivo lethality assay in a simple zoological organism, such as brine shrimp lethality test (BST) has been applied as a simple and useful tool for preliminary screening [8,13,29] of toxicity of physiologically active plant extracts[13,20,22] or synthesized compounds, detection of fungal toxins, heavy metal, pesticides and also cytotoxicity testing of dental materials[8,18]/ This general bioassay is rapid, reliable and has been used for over thirty years in toxicological studies[13]. However, it has been demonstrated that a positive relationship exists between brine shrimp lethality and human carcinoma. Thus, BST can also be extrapolated for cell line toxicity and anti tumor activity[5,15,16].

Principal of this method was based on the ability of certain compounds to kill laboratory cultured *Artemia* nauplii brine shrimp[19]. BST is one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive[13]. It has been shown that Artemia is highly vulnerable to toxins at the early developmental stages and assumed to exhibit their greatest sensitivity to test compounds[8]. Subsequently, in this study we used 24 h nauplii as object experimental.

Hence, in the present report, we synthesize chalcone (**1**) and its methoxy derivatives (**2,3**) by using Claisen-Schmidt condensation and catalyzed by NaOH 60%. The compounds were purified by recrystallization and characterized by ^{1}H-NMR, ^{13}C-NMR, IR, MS and UV-vis spectroscopic method. In addition, all the synthesized compounds were also examined their cytotoxic action by BST method (brine shrimp lethality test).

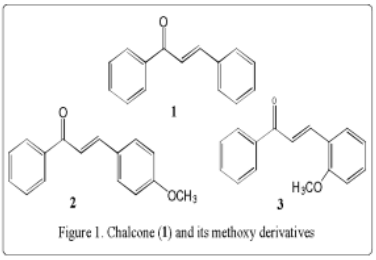


Figure 1. Chalcone (1) and its methoxy derivatives

Experimental Section

Materials and Instrumentation

All reagents and solvents used in this experimental were obtained from commercial sources as pro synthesis and pro analytical grade, such as acetophenone, benzaldehyde, anisaldehyde, *o*-methoxybenzaldehyde, sodium hydroxide, ethanol, n-hexane, ethyl acetate, chloroform, methanol and DMSO.

Melting points were measured with a Fisher John melting points apparatus without correction. Infrared (IR) spectra were recorded on a FT/IR- M 500 Buck Scientific Spectrophotometer and major absorptions are listed in wave number (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker 400 MHz, 5 mm probe instrument, and chemical shifts were reported in ppm on δ-scale from internal TMS. MS spectra were measured with a JEOL JMS D-600 spectrometer and using electro spray ionization (ESI) methods. Thin Layer Chromatography (TLC) was carried out on aluminium plates coated by silica gel GF254 (Merck), with eluent system n-hexane:ethyl acetate (7:1), and spot detection was performed with UV 254 nm. UV-visible spectra were measured with UV-Vis Hewlett Packard 8452A Spectrophotometer.

General procedure for synthesis chalcone and its methoxy derivatives

Equimolar quantities of acetophenone and corresponding aldehydes (benzaldehyde, anisaldehyde, *o*-methoxybenzaldehyde) 16.6 mmol, were mixed and dissolved in ethanol 12 ml. To this mixture, aqueous sodium hydroxide solution (60%, 1.5 ml) was dropped wisely and stirred occasionally for 15-105 minutes, at room temperature. Completion of the reaction was observed by TLC. After the completion of the reaction, the mixture was poured into crushed ice until form solid phase of products. The products were filtered with suction on Buchner funnel and washed with cold water until the washing are neutral to litmus. The crude chacones were dried in the open air for 30 minutes and purified by recrystallization with ethanol 96%. The products were identified by ^{1}H-NMR, ^{13}C-NMR, IR, MS and UV-vis spectroscopic method.

Procedure for Cytotoxicity test (Brine shrimp lethality test)

The artemia lethality assay was carried out according to Meyer with minor modifications[13,18]. Dried cysts or eggs of *Artemia sp* (about 30 mg) were placed into a hatching chamber, divided as dark and bright part, both containing sea water and kept under constant aerator for 24 hours[8]. After hatching, active nauplii free from egg shells were collected with Pasteur pipette from brighter part of chamber and ready to be used for the assay. Afterward, 5 mg of each synthesized compounds were accurately weighed and dissolved in 5 ml methanol to give stock solution with concentration of 10.000 ppm.

From the stock solution, a variety of solution concentrations were prepared as 0.25 ppm, 0.50 ppm, 1.00 ppm, 1.50 ppm, 2.00 ppm, 3.00 ppm and 5.00 ppm. Next, 5 ml each of these dosages were transferred into small vials and prepared in triplicate. The vials used for control experiment was stained with 1 ml methanol. All vials containing the dosages and the control were left overnight for the methanol to vaporize, leaving only the sample as residue.[4,18] To each of the vials containing the tested compounds (21 vials per sample), 2 drops of DMSO (max 1%) were added to redissolved the dosage followed by distilled sea water up to 5 ml. Then, 10 nauplii of *Artemia sp* were introduced into each of test vial using Pasteur pipette[4]. For the control test of each sample was added DMSO (1%) and sea water up to 5 ml. After 24 h incubation, the vials were observed using a magnifying glass, and followed by counting the numbers of survivors and calculating percentages of deaths[14]. Larvae were considered dead if they did not show any movement during several seconds of observation[8,14]. The resulting data were converted to probit analysis method for determination of the lethal dose 50% (LC_{50}) values for the tested compounds[11,21]. LC_{50} value greater than 1000 ppm for plant extracts was considered inactive[13], whereas LC_{50} values less than 30 ppm for pure compounds were considered toxic[12].

RESULTS AND DISCUSSION

The synthetic method of chalcone and its methoxy derivatives are as illustrated in Fig. 2. The conventional method Claisen schimdt condensation of acetophenone and commercially available benzaldehyde, anisaldehyde, *o*-methoxybenzaldehyde under NaOH 60% in ethanol proceeded smoothly to provide 1,3-diphenyl-2-propen-1-one (**1**, 73% yield), 3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (**2**, 93% yield) and 3-(2-methoxyphenyl)-1-phenylprop-2-en-1-one (**3**, 87% yield), respectively . The compounds were obtained as yellow needle crystals.

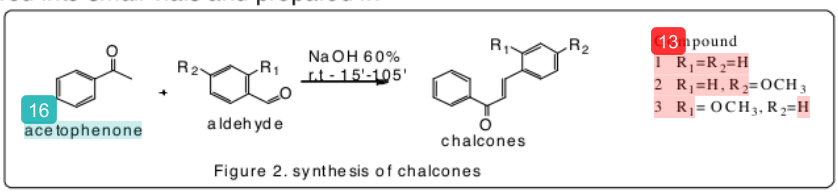


Figure 2. synthesis of chalcones

Sodium hydroxide in an aqueous solvent which used in this experiment leads to fast and reversible formation of intermediate compound. For that reason, it was used to increase the reaction rate of Claisen-Schmidt condensation. Base removed an acidic alpha hydrogen from acetophenone, producing a resonance-stabilized enolate ion[17]. This enolate ion then attacked aldehyde molecule, yielding a neutral condensation product and followed by dehydration to generate chalcones in good yields.

All the compounds provided a single spot in TLC analysis and possessed a very sharp melting range, therefore it can be concluded that the synthesized compounds were pure. From IR spectra analysis, all the synthesized compounds have a sharp absorbance at around 1660 cm^{-1} which showed the presence of carbonyl group (C=O) conjugated with phenyl group and around 1600 cm^{-1} which assumed the presence of alkene group (C=C) of aromatic. Sharp absorbance also exhibited about 980 cm^{-1} which supposed to owe double bond (C=C) in *trans* position. Additionally, chalcone 2 and 3 have sharp absorbance at around 1200 cm^{-1} which mentioned the presence of methoxy group of ethers (aryl-O-CH_3).

In ^{1}H-NMR spectrum, the peak presence of protons at olefinic double (Hα,β of unsaturated carbonyl group) were exhibit at δ 7.51 ppm, 7.79 ppm with coupling constant 15.6 Hz on chalcone's NMR spectrum; 7.41 ppm, 7.78 ppm with coupling constant 15.6 Hz on chalcone derivative 2's NMR spectrum and 7.61 pmm, 8.13 ppm with coupling constant 15.6 Hz and 16.0 Hz on chalcone derivative 3's NMR spectrum. From the reference, coupling constant for protons in olefinic double bond with *trans* position is 12-18 Hz, whereas *cis* position is 6-12 Hz. Accordingly, it can be concluded that entirely synthesized compounds have olefinic protons double bond at *trans* position. The peak pattern of compound 2 assigned has *para* substituent and compound 3 has *ortho* substituent, which is similar to those mentioned in the reference.[28] Both compound 2 and 3 possessed methoxy group which appeared at δ 3.84 ppm. However, others characterization analysis has confirmed the structure of the formed products.

Characterization data of the synthesized compounds were described as below:

1,3-diphenyl-2-propen-1-one (1)

Yellow crystals (2.5 g, 12.2 mmol, 73.6%), m.p= 54 °C. Spectral data: UV-vis (λ_{max}, ethanol, nm): 228, 308. IR (KBr, cm^{-1}): 1661 (C=O), 1604 (CH=CH olefinic), 1574 and 1447 (C=C aromatic), 12[illegible]6 (C-O).^{1}H-NMR ($CDCl_3$-*d*, δ, ppm): 7. 33-7.[illegible]28 (3H, m, Ar-H), 7.42-7.48 (2H, m, Ar-H'), 7.51 (1H, d, *J* = 15.6 Hz, H_α), 7.50-7.56 (1H, m, Ar-H'), 7.57-7.64 (2H, m, Ar-H), 7.79 (1H, d, *J* = 15.6 Hz, H_β), 7.96-8.04 (2H, dd, *J*= 1.2 Hz and 8.4 Hz, Ar-H'). ^{13}C-NMR ($CDCl_3$-*d*, δ, ppm): 121.7, 128.2 (2C), 128.3 (2C), 128.4 (2C), 128.7 (2C), 130.3, 132.6, 134.6, 137.9, 144.6, 190.2. MS (m/z): ESI 209 [M^++H, $C_{15}H_{13}O$]. Rf (n-hexane:ethyl acetate = 7:1)= 0.76.

3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (2)

Yellow crystals (3.8 g, 15.9 mmol, 96.2%), m.p= 69 °C. Spectral data: UV-vis (λ_{max}, ethanol, nm): 242, 342. IR (KBr, cm^{-1}): 1657 (C=O), 1599 (CH=CH olefinic), 1576 and 1511 (C=C aromatic), 1170 (C-O aliphatic), 1213 and 1017 (C-O aromatic).^{1}H-NMR ($CDCl_3$-*d*, δ, ppm): 3.84 (3H, CH_3), 6.87-6.99 (2H, m, Ar-H), 7.41 (1H, d, *J* = 15.6 Hz, H_α), 7.46-7.52 (2H, m, Ar-H'), 7.53-7.58 (1H, m, Ar-H'), 7.59-7.64 (2H, m, Ar-H), 7.78 (1H, d, *J* = 15.6 Hz, H_β), 7.96-8.07 (2H, m, Ar-H'). ^{13}C-NMR ($CDCl_3$-*d*, δ, ppm): 55.3, 114.3 (2C), 119.6, 127.5, 128.3 (2C), 128.5 (2C), 130.2 (2C), 132.5, 138.4, 144.6, 161.6, 190.5. MS (m/z): ESI 239 [M^++H, $C_{16}H_{15}O_2$]. Rf (n-hexane:ethyl acetate = 7:1)= 0.22.

3-(2-methoxyphenyl)-1-phenylprop-2-en-1-one (3)

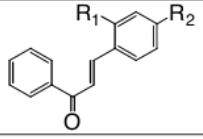
Yellow crystals (3.3 g, 13.8 mmol, 83.4%), m.p= 56 °C. Spectral data: UV-vis (λ_{max}, ethanol, nm): 298, 346. IR (KBr, cm^{-1}): 1661 (C=O), 1601 (CH=CH olefinic), 1574 (C=C aromatic), 1179 (C-O aliphatic), 1211 and 1016 (C-O aromatic).^{1}H-NMR ([illegible]Cl_3-*d*, δ, ppm): 3.84 ([illegible]s, CH_3), 6.88 (1H, d, *J*= 8.4 Hz, Ar-H), 6.95 (1H, t, *J* = 7.6 Hz, Ar-H), 7.29-7.36 (1H, m, Ar-H), 7.

2-7.48 (2H, m, Ar-H'), 7.49-7. 56 (1H, m, Ar-H), 7.6 1H, d, J = 15.6 Hz, H_α), 7.58-7.65 (1H, m, Ar-H'), 7.98-8.04 (2H, m, Ar-H'), 8.13 (1H, d, J = 16.0 Hz, H_β). ^{13}C-NMR ($CDCl_3$-*d*, δ, ppm): 55.2, 111.0 (2C), 120.5, 122.4, 123.5, 128.2 (2C), 128.3 (2C), 128.9, 131.6, 132.4, 138.3, 140.11, 158.5, 190.7. MS (m/z): ESI 261 [M^++Na, $C_{16}H_{14}O_2Na$]. Rf (n-hexane:ethyl acetate = 7:1)= 0.29.

Additionally, the compounds synthesized in this work are carried on to toxicity bioassay against *Artemia sp.* The Brine shrimp lethality assay is regarded as one of the most useful biological tests to accomplish further development to discover antitu r compounds. As this bioassay also has good correlation with the human solid tumor cell lines.

All compound showed a dose dependent cytotoxic activity at the tested concentrations as illustrated in fig. 3. The LC_{50} value less than 30 ppm was considered noteworthy toxic for pure compounds. The LC_{50} results of halcone and its two methoxy derivatives evaluated in this screening are listed in table 1. The *para*-methoxy chalcone (**2**) was the most active than other compounds (**1** and **3**), presenting the lowest LC_{50} of 6.33 ppm, while chalcone (**1**) and the *ortho*-methoxy chalcone gave LC_{50} 6.35 ppm and 7.75 pmm, respectively. From this result, it can be concluded that all the synthesized products showed significant lethality against brine shrimp. Along with, the *ortho*-methoxy substituted chalcone (**3**) has steric hindrance to the receptor of *Artemia sp.*, so reducing its ability to kill *Artemia* nauplii compared to its *para*-methoxy substituted chalcone (**2**). In spite of this, all examined compounds can be regarded as prosperous candidate for antitumor agents. Further and more specific bioassays are on progress and will be published shortly in the future.

Table 1: Brine Shrimp Lethality Assay of Chalcone and Its Methoxy Derivatives

Structure	Compound	LC_{50} (ppm)	Substituents	
			R_1	R_2
	1	6.35	H	H
	2	6.33	H	OCH_3
	3	7.78	OCH_3	H

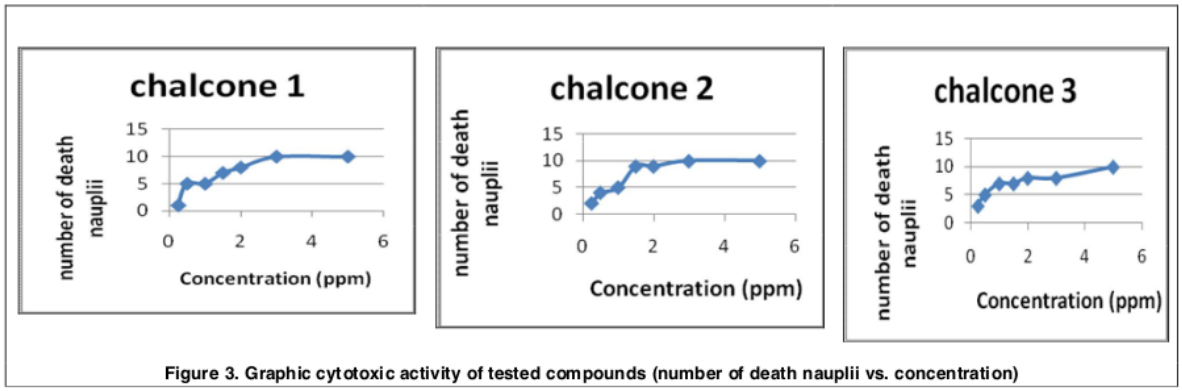


Figure 3. Graphic cytotoxic activity of tested compounds (number of death nauplii vs. concentration)

CONCLUSIONS

Chalcone and its methoxy derivatives have been synthesized by a classical method, Claisen-Schmidt condensation in good yields. Characterization analysis has confirmed structure of the formed products.

The present study also revealed that 3 synthesized compounds are toxic against *Artemia sp.* Therefore, these compounds should be studied furthermore for getting antitumor compounds.

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