World Journal of Chemistry 4 (1): 09-14, 2009
ISSN 1817-3128

Synthesis and Antimicrobial Studies of (*E*)-3-(4-Alkyloxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one, (*E*)-3-(4-Alkyloxyphenyl)-1-(4-Hydroxyphenyl)prop-2-en-1-one and their Analogues

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Abstract: A series of (*E*)-3-(4-alkyloxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one **(2a-c)** and (*E*)-3-(4-alkyloxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one **(3a-c)** have been synthesized *via* Claisen-Schmidt condensation. The compounds differ in the length of alkyl groups, C_nH_{2n+1}, where n= 10, 12 and 14. The structures of the synthesized compounds were defined by elemental analysis, IR, ^1H and ^{13}C NMR. Antimicrobial studies were carried out against *E. coli* ATCC 8739 to evaluate the effect of the hydroxyl and alkyl groups of the synthesised chalcones. All the synthesized compounds have shown significant antimicrobial activities. Chalcones **(2a-c)** showed better antimicrobial activities compared to chalcones **(3a-c)** respectively, with (*E*)-3-(4-decyloxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one showed the highest antimicrobial activity among the compounds tested.

Key words: Chalcones · Hydroxyl group · Alkyl chains · Antimicrobial activities

INTRODUCTION

Chalcone is a common natural pigment and one of the important intermediate in the biosynthesis of flavonoid. Synthetic and naturally occurring chalcones have been extensively studied and developed as one of the pharmaceutically important molecules. Chalcones has been reported to possess broad spectrum of biological properties such as an anticancer [1,2], antimalarial activities [3], anti-inflammatory [4], antioxidant and antimicrobial activity [5], antiplatelet activity [6], antiangiogenic and antitumour [7], as well as antihyperglycemic [8]. One of the most convenient and applied methods to synthesize chalcone is *via* Claisen-Schmidt condensation, which involves cross aldol condensation of appropriate benzaldehyde and acetophenone in presence of base as catalyst.

Many conditions have been employed in synthesizing chalcones due to ease of chalcone structure itself to be substituted [1,9]. The arrangement of hydroxyl groups as the substituents on chalcones was claimed to be vital in antimicrobial studies [10-12]. Apart from hydroxyl groups, the effect of hydrocarbon chain-length has also been reported to contribute in antimicrobial activity. It was envisaged that different length of hydrocarbon chains would produce lipophilic properties to disrupt microorganisms' cell wall [13].

In this paper, we report on the synthesis of (*E*)-3-(4-alkyloxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one **(2a-c)** and (*E*)-3-(4-alkyloxyphenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one **(3a-c)** possessing alkyl chains of varying length from C_{10} to C_{14}. The antibacterial study of chalcone derivatives was performed towards *E. coli* ATCC 8739 to evaluate the effect of hydroxyl group arrangement at the *ortho* and *para* position as well as the optimum length of the alkyl chain in the synthesized chalcones.

MATERIALS AND METHODS

Materials: 4-hydroxybenzaldehyde, 4-hydroxyacetophenone and 1-bromoalkanes were obtained from Merck Company and used without further purification. All other reagents and solvent were used as received.

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Measurements: Melting points were determined by the open tube capillary method and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 1605 FTIR Spectrophotometer using neat liquid film and nujol mull. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL ECA 500 at 500 MHz with the chemical shits (δ, ppm) reported relative to $CDCl_3$ as a standard reference. Flash column chromatography was performed at atmospheric pressure using Malinckrodt Silica Gel 60, (230-400 mesh) as a stationary phase. All chemicals and solvents obtained were used without further purification.

4-decyloxybenzaldehyde (1a) [14]**:** A mixture of 4-hydroxybenzaldehyde (6.11 g, 50 mmol), K_2CO_3 (8.29 g, 60 mmol), bromodecane (12.40 mL, 60 mmol) and TBAI (1.85 g, 5 mmol) in MEK (150 mL) was heated at reflux for 12 h. The mixture was filtered and cooled at room temperature. Water (30 mL) was added to the filtrate and the layers separated. The aqueous layer was extracted with dichloromethane (2 x 30 mL). The combined layers were washed with water (2 x 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude was purified by column chromatography (eluting with 1:20 ethyl acetate/petroleum ether) to afford **1a** (8.69 g, 66%) as a viscous brown oil. The FTIR and NMR data were consistent with the reported literature [15]. The same general procedure gave compounds **1b-c**, with the scale (mL, mmol, [bromoalkane]) and yields given below.

4-dodecyloxybenzaldehyde (1b): Bromododecane (14.38 mL, 60 mmol). Yield: 13.03g, 90%. The FTIR and NMR data were consistent with the reported literature [15].

4-tetradecyloxybenzaldehyde (1c): Bromotetradecane (16.31 mL, 60 mmol). Yield: 11.78g, 74%. The FTIR and NMR data were consistent with the reported literature [15].

(*E*)-3-(4-decyloxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (2a): A mixture of 2-hydroxyacetophenone (2.72 ml, 20mmol) and **1a** (5.25 ml, 20 mmol) in 60 ml of methanol was added onto a solution of KOH (4.04 g, 72 mmol) in methanol (10 ml). The mixture was heated at reflux for 10 h. The reaction was cooled to room temperature and acidified with cold diluted HCl (2N). The resulting precipitate was filtered, washed and dried. The crude was recrystallized from hexane to give **2a** (4.35 g, 58 %) as yellow crystals, m.p. 85-85.1°C; Found: C, 78.47: H, 8.20%. Calcd for $C_{25}H_{32}O_3$: C, 78.91: H, 8.48%; ν_{max} (nujol mull/cm^{-1}) 3070, 2922, 2853, 1648, 1592, 994, 765. δ_H (500MHz, $CDCl_3$) 0.87 (3H, t, 1xCH_3), 1.26-1.79 (16H, m, 8xCH_2), 4.00 (2H, t, 1xCH_2), 6.91-6.96 (1H, m, Ar-H), 6.93 (2H, d, *J* 8.55, Ar-H), 7.01 (1H, d, *J* 8.55, Ar-H), 7.47 (1H, dd, Ar-H), 7.53 (1H, d, *J* 15, 1x olefinic H), 7.61 (2H, d, *J* 8.55, Ar-H), 7.90 (1H, d, *J* 15, 1x olefinic H), 7.91 (1H, d, Ar-H). δ_C(500 MHz, $CDCl_3$) 14.09, 22.64, 25.95, 29.09, 29.28, 29.33, 29.52, 30.90, 31.86, 68.21, 114.96, 117.31, 118.54, 118.70, 120.09, 127.04, 129.48, 130.53, 136.08, 145.46, 161.66, 163.50, 193.65. The same general procedure gave compounds **2b-c**, with the scale (mmol, mL [**1b-c**]) and yields given below.

(*E*)-3-(4-dodecyloxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (2b): 1b (5.81 ml, 20 mmol). Yield (4.14 g, 50%) as yellow crystals, m.p: 82.5°C; Found: C, 79.08: H, 8.76%. Calcd for $C_{27}H_{36}O_3$: C, 79.37: H, 8.88%; ν_{max} (nujol mull/cm^{-1}) 3059, 2920, 2851, 1637, 1605, 991, 764. δ_H (500MHz, $CDCl_3$) 0.86 (3H, t, 1xCH_3), 1.27-1.78 (20H, m, 10CH_2), 4.00 (2H, t, 1xCH_2), 6.91-6.95 (1H, m, Ar-H), 6.93 (2H, d, *J* 8.60, Ar-H), 7.01 (1H, d, *J* 8.60, Ar-H), 7.48 (1H, dd, Ar-H), 7.53 (1H, d, *J* 15, 1x olefinic H), 7.61 (2H, d, *J* 9.15, Ar-H), 7.90 (1H, d, *J* 15, 1x olefinic H), 7.91 (1H, d, Ar-H). δ_C (500 MHz, $CDCl_3$) 14.13, 22.69, 26.00, 29.13, 29.35, 29.56, 29.59, 29.64, 29.66, 30.95, 31.92, 68.25, 114.99, 117.35, 118.57, 118.75, 120.13, 127.07, 129.55, 130.57, 136.13, 145.50, 161.69, 163.54, 193.69.

(*E*)-3-(4-tetradecyloxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (2c): 1c (6.37 ml, 20 mmol). (5.53 g, 64 %) as yellow crystals, m.p: 83.4°C; Found: C, 79.60: H, 9.10%. Calcd for $C_{29}H_{40}O_3$: C, 79.77: H, 9.23%); ν_{max} (nujol mull/cm^{-1}) 3065, 2924, 2853, 1636, 1604, 990, 765. δ_H (500MHz, $CDCl_3$) 0.88 (3H, t, 1xCH_3), 1.24-1.78 (24H, m, 12xCH_2), 4.00 (2H, t, 1xCH_2), 6.91-6.93 (1H, m, Ar-H), 6.93 (2H, d, *J* 8.60, Ar-H), 7.01 (1H, d, *J* 8.6, Ar-H), 7.47 (1H, dd, Ar-H), 7.54 (1H, d, *J* 15, 1x olefinic H), 7.61 (2H, d, *J* 8.60, Ar-H), 7.90 (1H, d, *J* 15, 1x olefinic H), 7.91 (1H, d, Ar-H). δ_C (500 MHz, $CDCl_3$) 14.13, 22.68, 25.98, 29.12, 29.36, 29.58, 29.64, 31.91, 68.23, 114.96, 117.30, 118.56, 118.72, 120.11, 127.50, 129.50, 130.55, 136.10, 145.48, 161.66, 163.51, 193.66.

(*E*)-3-(4-decyloxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (3a): A mixture of 4-hydroxyacetophenone (2.72 g, 20mmol) and **1a** (5.25 mL, 20 mmol) in 60 mL of methanol was added under stirring to a solution of KOH (4.04 g, 72 mmol) in methanol (10 mL). The mixture was heated at reflux for 10 h. The reaction was cooled to room temperature and acidified with cold diluted HCl (2N). The resulting precipitate was filtered, washed and dried. The crude was recrystallized from hexane: ethanol (7:1) to give **3a** (6.83 g, 54%) as yellow crystals. The FTIR and NMR data were consistent with the reported literature [15]. The

same general procedure gave compounds **3b-c**, with the scale (mmol, mL [**1b-c**]) and yields given below.

(*E*)-3-(4-dodecyloxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (3b): 1b (5.81 mL, 20 mmol). Yield: 8.32 g, 52%. The FTIR and NMR data were consistent with the reported literature [15].

(*E*)-3-(4-tetradecyloxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (3c): 1c (6.37 g, 20 mmol). Yield: 5.51 g, 54%. The FTIR and NMR data were consistent with the reported literature [15].

Antibacterial Screening: The antibacterial activities of the synthesized compounds were studied against *E. coli* ATCC 8739 using turbidimetric kinetic method. The inoculums were allowed to grow on media containing nutrient broth at 37°C with permanent stirring at 250 rpm for 18 h. 10 ml of culture medium with increasing concentration of the compounds dissolved in propanol were inoculated with 0.2 ml of inoculums and the mixture was shaking at 250 rpm at 37 °C. Inoculums with solvent were used as control. Aliquots of each replicate were taken every 1 h interval for 6 h and the transmittance (T) were registered in a UV-Visible spectrophotometer Optima SP-300. The antibacterial activity was determined by graph as ln Nt which related to the number cfu/ml (colony forming units/ml) for *E. coli* versus time [16].

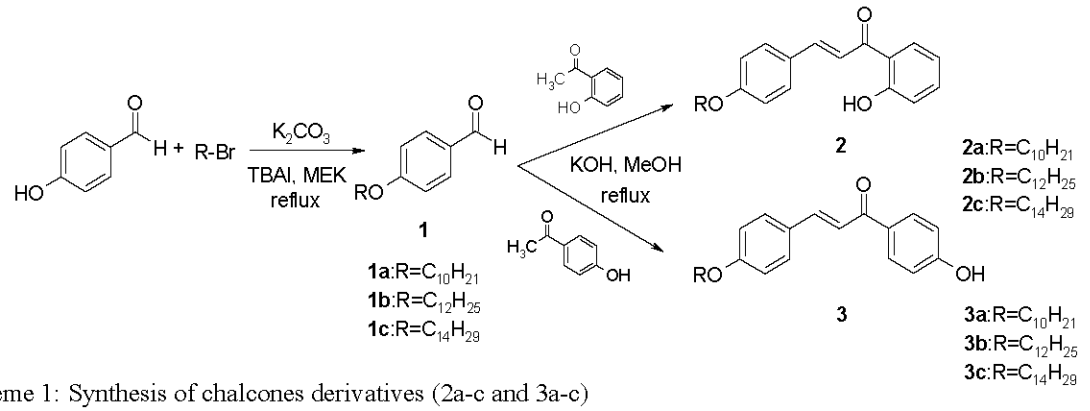
RESULTS AND DISCUSSION

Chemistry: Preparation of alkoxybenzaldehyde prior to chalcone synthesis is depicted in Scheme 1. **1a-c** was synthesized by refluxing 4-hydroxybenzaldehyde with a series of bromoalkanes (C_{10}, C_{12} and C_{14}) in the presence of K_2CO_3 and TBAI in methyl ethyl ketone. IR showed the presence of new bands which attributed to ν_{CH2} at 2960-2850 cm^{-1} and strong bands which attributed to $\nu_{C=O}$ at 1627-1693 cm^{-1}. The presence of aliphatic carbon chainwas shown in the region of 0.70 -0.90 ppm and 4.06-4.30 ppm in ^{1}H NMR spectra whereas HC=O was observed at 9.81-9.90 ppm. ^{13}C NMR spectra revealed the presence of aliphatic carbon chain at 13-32 ppm which was consistent with the proposed structure.

Compounds **1a-c** were further reacted with 2-hydroxyacetophenone *via* the Claisen-Schmidt condensation in methanol reflux to afford **2a-c** as yellow crystals. IR spectral showed the presence of ν_{CH2} band at 2851-2922 cm^{-1} and $\nu_{C=O}$ stretching frequenciy at 1636-1648 cm^{-1}. The absorption bands observed at 991-994 $cm^{-1}$was due to *trans* double bond. The presence of bands at region of 748-765 $cm^{-1}$was attributed to *ortho* disubstituted benzene. The chemical structures of all synthesized compounds were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopic methods and showed the appearing peaks corresponded to the structures. ^{1}H NMR spectra showed the presence of vinylic proton at 7.53 and 7.90 ppm with a coupling constant, J_{ab} 15 MHz referred to *trans* conformation. In the ^{13}C NMR spectra, C=O and COH were observed at 193.63-193.69 and163.50-163.54 respectively.

Compounds **3a-c**, on the other hand, were obtained by condensation of 1**a-c** with 4-hydroxyacetophenone following the same procedures. IR spectral showed the presence of ν_{CH2} bands at 2849-2922 $cm^{-1}$and $\nu_{C=O}$ bands at 1636-1648 cm^{-1}. The *trans* double bond was observed at 986 cm^{-1} while bands appeared at region of 824-825 $cm^{-1}$was attributed to *para* disubstituted benzene. ^{1}H NMR showed the formation of *trans* vinylic proton at 7.39-7.41 and 7.67-7.77 respectively with a coupling constant, J_{ab} 15 MHz.

Antibacterial Activities: Result on the inhibition activity of compound **2a-c** and **3a-c** against *E. coli* is shown in Figure 1. All of the synthesized compounds exhibited



Scheme 1: Synthesis of chalcones derivatives (2a-c and 3a-c)

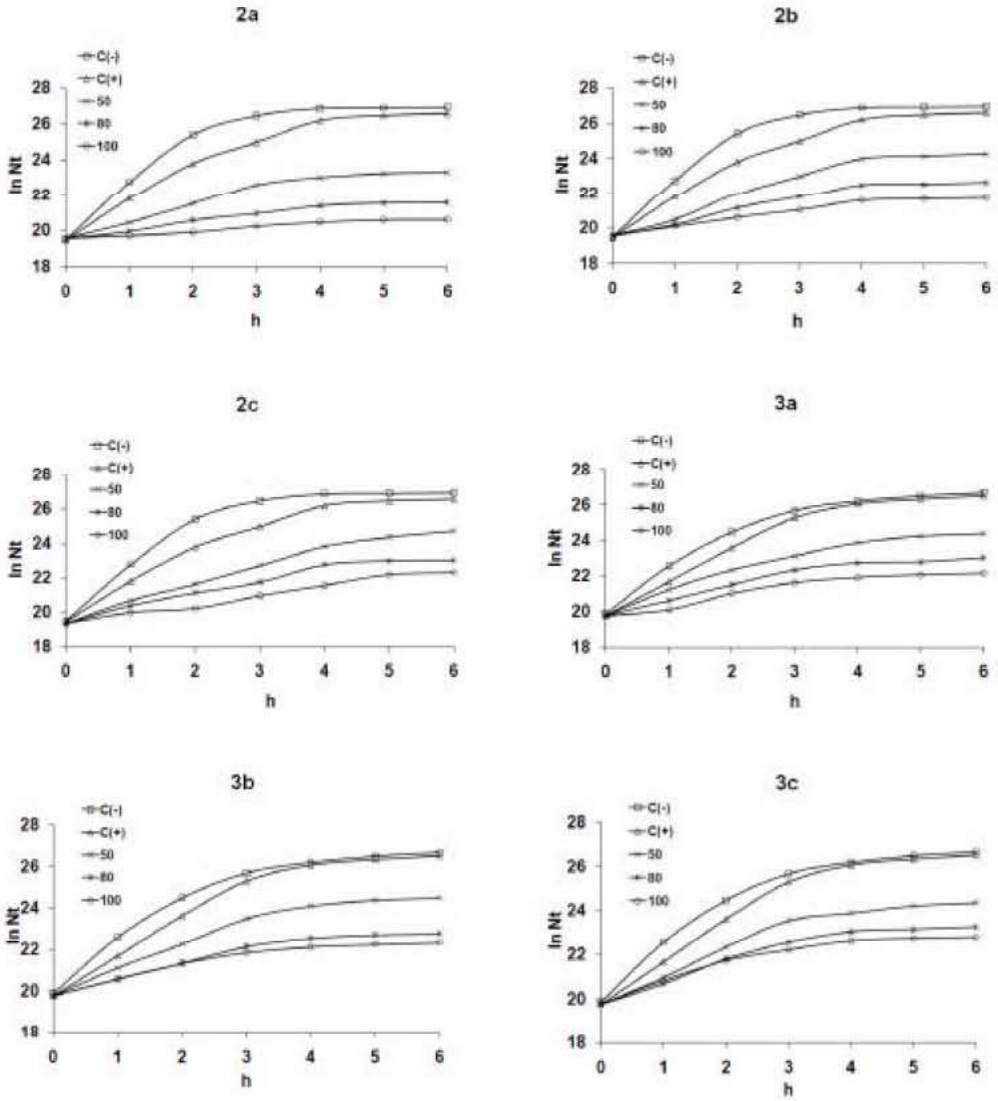


Fig. 1: Inhibition activity of 2a-c and 3a-c against *E. coli* shown as ln Nt for *E. coli* growth versus time. The analogues 2a-c and 3a-c are as indicated in the main text

bacteriostatic activities upon introduction at different concentrations of 50 ppm, 80 ppm and 100 ppm. Compound **2a** showed almost complete inhibition at 100 ppm compared to compound **2b**, **2c** and **3a-c**. Different effect of the synthesized chalcones at various concentrations can be further shown by their minimum inhibitory concentrations (MIC). The MIC of these compounds were determined by extrapolating the concentration at the zero growth rate of *E. coli* (μ=0) [16]. The series for MIC observed was **2a** (114.5 ppm) <**2b** (143 ppm) <**3a** (155.4 ppm) <**3b** (155.5 ppm) <**2c** (170.5 ppm) <**3c** (175.8 ppm) (Figure 2).

These studies showed that amongst (*E*)-3-(4-alkyloxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one, compound **2a** exhibited the best bacteriostatic activities followed by **2b** and **2c**. Similarly, analogues of (*E*)-3-(4-alkyloxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one showed that compound **3a** exhibited the best bacteriostatic activities followed by **3b** and **3c**. These results indicated that hydroxyl groups in all the synthesized compounds play an important role to exhibit bacteriostatic activities. The hydroxyl group at the *ortho* positions, which is closer to the carbonyl region, showed better activities compared to hydroxyl group at the *para* position.

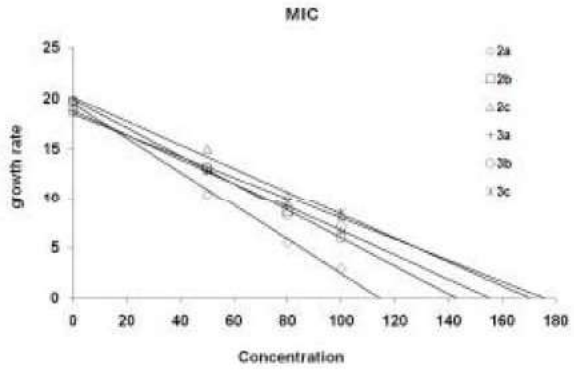


Fig. 2: Minimum inhibitory concentrations of compounds 2a-c and 3a-c determined by extrapolating the concentration at the zero growth rate of *E. coli* (μ=0) [16].

Upon introduction of different alkyl chains, both compound **2a-c** and **3a-c** having C_{10}, C_{12} and C_{14} alkyl groups respectively showed decreasing bacteriostatic activities with the increase in the alkyl chains. This phenomenon indicated the length of alkyl chain in the different compound had a significant influence in giving the optimum antimicrobial activities [13].

CONCLUSION

New homologues series of chalcone derivatives as antimicrobial agents have been successfully synthesized. All the synthesized chalcones exhibited promising antibacterial activities against *E. coli* where the presence of hydroxyl groups at the *ortho* position showed better antimicrobial activities compared to *para* position. Besides hydroxyl groups, the introduction of alkyl chains (C_{10}, C_{12} and C_{14}) was also contributing to the antibacterial activities where the inhibition activity is observed to be concentration dependent.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Malaysia Sarawak for financing this project through UNIMAS grant DI/01/2007(01).

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