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SHORT COMMUNICATION

Synthesis and Antibacterial Study of Aspirin-Chalcone Derivatives

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ABSTRACT

The chemistry of aspirin and chalcone derivatives has been extensively studied and developed as one of the pharmaceutically important molecules. In this study, new aspirin-chalcone derivatives have been successfully synthesized and characterized *via* FTIR, ^{1}H and ^{13}C NMR spectroscopy. The antibacterial activities of synthesized compounds were investigated towards *Escherichia coli* ATCC 8739 *via* turbidimetric kinetic method. The newly synthesized aspirin-chalcone derivatives, however showed poor antibacterial activity against *E. coli* ATCC 8739 at the concentration of 50, 80 and 100 ppm. The effect of the molecular structure of the synthesized compounds on the antibacterial activity is discussed.

Keywords**:** Aspirin, chalcone, antimicrobial, *E. coli*

Aspirin is a well-known salicylate drug, which has been used as an analgesic and anti-inflammatory medication. Modifications of aspirin have been carried out widely and many aspirin derivatives were reported to show various biological activities such as antibacterial (Al-Bakri *et al*. 2009), antithrombic and antiplatelet (Lechi *et al*., 1996) and also anticancer properties (Lechi *et al*., 1996; Zheng *et al*., 2007).

Our group has recently reported on the incorporation of aspirin with thiourea moiety with excellent antibacterial property against *E. coli* (Ngaini *et al*., 2012). Besides thiourea, chalcones which belong to the flavonoid family has also been identified as an interesting compound to display a diverse array of pharmacological activities. Chalcones show many biological properties including anticancer, antimalaria, antimicrobial, anti-inflammatory and antibacterial (Hsieh *et al.*, 1998; Ram *et al.*, 2000). Novel 2,4,2'-trihydroxy-5-methylchalcone, for instance, was reported to inhibit the growth of different Gram-positive bacteria (Sato *et al.*, 1996)

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This finding has stimulated our interest in the synthesis of a series of chalcone compounds containing aspirin moiety. In this paper, we report on the synthesis of aspirin-chalcone compounds **2a-b** *via* incorporation of hydroxychalcone onto aspirin moiety. The hydroxychalcones **1a-b** were earlier prepared *via* Claisen-Schmidt condensation prior to incorporation onto aspirin derivatives. The antibacterial property of the synthesized aspirin-chalcone derivatives were also studied against wild-typed *E. coli* ATCC8739.

Aspirin, oxalyl chloride, 4-hydroxybenzaldehyde, acetophenone, benzaldehyde and 4-hydroxyacetophenone were obtained from Merck and used without further purification. All the other reagents and solvents were used as received.

Measurements: Melting points were determined by the open tube capillary method and are uncorrected. Infrared (IR) spectra (ν/cm^{-1}) were recorded as KBr pellets on a Perkin Elmer 1605 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA 500 spectrometer at 300 MHz (^{1}H)

and 125 MHz (^{13}C) with the chemical shifts δ (ppm) reported relative to $CDCl_3$-D_6 as standards.

Preparation of (*E*)-3-(4-hydroxyphenyl)-1-phenylpropenone, 1a

A mixture of KOH (3.0 g, 54.0 mmol), 4-hydroxybenzaldehyde (1.46 g, 12.0 mmol) and acetophenone (1.44 g, 12.0 mmol) was dissolved in 95% ethanol (30 mL) and stirred at room temperature for 18 h. The mixture was cooled in an ice bath and hydrochloric acid (8 M) was added dropwise to form a light yellow solid. The precipitates were filtered, washed with water and recrystallize in hot ethanol. It is then air-dried to give the title compound **1a** (0.94 g, 35%) as a yellow crystal. m.p. 181.5 ºC (Petrov *et al*., (2008), , 181-182 ºC); υ_{max} (thin film/cm^{-1}) 3220 (OH), 1650 (C=O), 1599 (aromatic); δ_H (300 MHz, MeOD) 4.92 (H, s, O-H), 6.86 (2H, d, *J* = 8 Hz, 3'-H), 8.04 (2H,d, *J* = 8 Hz, 2'-H), 7.57 (H, d, *J* = 15 Hz, H-α), 7.79 (H, d, *J* = 15 Hz, H-β) 7.55-7.65 (5H, m, 2''-H to 4''-H); δ_c (75.5 MHz, MeOD) 115.6, 118.4, 126.3, 128.1, 128.4, 130.5, 132.6, 138.3, 145.7, 160.3, 191.5.

Synthesis of (*E*)-1-(4-hydroxyphenyl)-3-phenylpropenone, 1b

A mixture of KOH (3.0 g, 54.0 mmol), 4-hydroxyacetophenone (1.63 g, 12.0 mmol) and benzaldehyde (1.2 ml, 12.0 mmol) was dissolved in 95% ethanol (30 mL) and stirred at room temperature for 18 h. The mixture was cooled in an ice bath and hydrochloric acid (8 M) was added dropwise to form a light yellow solid. The precipitates were filtered, washed with water and recrystallize in hot ethanol. It is then air-dried to give the title compound **1b***:* (1.02 g, 38%) as a yellow solid; m.p. 177.8 ºC (Gul *et al*., (2008) 174 ºC); υ_{max} (thin film/cm^{-1}) 3128 (OH), 1646 (C=O), 1591 (aromatic); δ_H (500 MHz, MeOD) 6.86 (2H, d, *J* = 7 Hz, 3'-H), 8.00 (2H, d, *J* = 7 Hz,2'-H), 7.49 (2H, d, *J* = 7 Hz, 2''-H), 7.53 (2H, d, *J* = 7 Hz, 3''-H), 7.49 (1H, t, 1'-H),7.26 (1H, d, *J* = 15 Hz, H-α), 7.56 (1H, d, *J* = 15 Hz, H-β) ; δ_c (77 MHz, MeOD) 114.2, 115.1, 121.9, 126.9, 130.8, 131.8, 144.8, 160.0, 189.7.

General procedure for synthesis of aspirin-chalcone derivatives 2a-b

Oxalyl chloride (0.43 mL, 5.0 mmol) in distilled DCM (20 mL) was added dropwise into aspirin (0.90 g 5.0 mmol) in 20 mL distilled DCM. A few drops of DMF were added to initiate the reaction. The reaction was stirred for about 1 h under N_2 atmosphere. Chalcone **1a-b** (0.73g, 5.0 mmol) and triethylamine (0.70 mL, 5.0 mmol) was added and the mixture was heated at reflux for 5 h. The mixture was cooled to room temperature and filtered. The crude was recrystallized from ethyl acetate to give the aspirin-chalcone derivatives **2a-b.**

[4-[(*E*)-3-phenylprop-2-enoyl]phenyl] 2-acetoxybenzoate (2a): Compound **2a** was obtained as white powder. Yield: 1.12g (57%); m.p. 135° C; R_f 0.39 (1:3 hexane: tetrahydrofuran); IR (KBr pellet) υ_{max} cm^{-1}: 2854 (C-H stretch of CH_3), 1736 (C=O stretch of ester), 1660 and 1606 (C=O and C=C stretch of α,β-unsaturated ketone). ^{1}H NMR δ(ppm) (300 MHz, $CDCl_3$) 2.95 - 3.09 (3H, t, 2'''-H), 7.49 (1H, d, *J* = 15.4 Hz, H-α), 7.80 (1H, d, *J* = 15.45 Hz, H-β) and 7.06 – 7.83 (13H, m,3-H to 6-H, 2'H-to 3'-H, 2''-H to 4''-H). ^{13}C NMR δ(ppm) (125 MHz, $CDCl_3$): 8.6, 121.7, 122.0, 128.5, 130.2, 130.6, 132.2, 145.2, 151.3, 154.0, 162.4, 169.7, 189.3.

4-[(*E*)-3-oxo-3-phenyl-prop-1-enyl]phenyl] 2-acetoxybenzoate (2b): Compound **2b** was obtained as white crystal. Yield: 0.99g (53%); m.p. 136 °C; R_f 0.88 (1:3 hexane: tetrahydrofuran); IR (KBr pellet) υ_{max} cm^{-1}: 2854 (C-H stretch of CH_3), 1736 (C=O of ester), 1660 and 1606 (C=O and C=C of α,β-unsaturated ketone). ^{1}H NMR δ(ppm) (300 MHz, $CDCl_3$): 3.02-3.05 (3H, t, H-2''), 7.34(1H, d, *J*=15.4, H-α), 7.44 - 8.07 (13H, m,3-H to 6-H, 1'H-to 3'-H, 2''-H to 3''-H) and 8.19 (1H, d, *J*=15.4, H-β). ^{13}C NMR δ(ppm) (125 MHz, $CDCl_3$): 8.4, 121.9, 122.25, 124.2, 126.5, 128.9, 130.4, 131.9, 144.5, 153.7, 162.3, 169.2, 188.1.

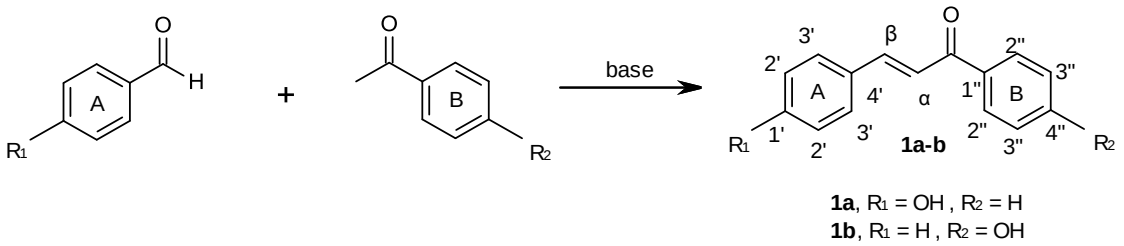
Antibacterial screening

The antibacterial activities of the synthesized compounds were studied against *E. coli* ATCC 8739 by using turbidimetric kinetic method (Devia *et al.*, 1998) The inoculums were allowed to grow on media which contain nutrient broth at 37 ºC with permanent stirring at 250 rpm for 18 h. 10 ml of culture medium with increasing concentration of the

compounds dissolved in DMSO were inoculated with 0.2 ml of inoculums and the mixture was shaken at 250 rpm at 37 ºC. The solvent was used as control. Aliquots of each replicate were taken at every 1 h interval for 7 h and the transmittance (T) was 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.

Chemistry: The preparations of chalcone **1a-b** are shown in Scheme 1. All chalcone derivatives **(1a-b)** were synthesized *via* the base catalyzed Claisen–Schmidt condensation reaction in methanol with yields ranging from 35–38%. The relatively low yield was attributed to trace amount of side products of Cannizzaro reaction or ketone auto condensation (Climent *et al.*, 2004; Calvino *et al*., 2006). The IR spectra showed the presence of ν_{OH} at 3467-3468 cm^{-1} while strong bands attributable to $\nu_{C=O}$ appeared at 1660 cm^{-1}. The chemical structures of all compounds were confirmed by ^{1}H- and ^{13}C-NMR spectroscopic methods and showed the peaks that corresponded to the structures. From the ^{1}H-NMR spectra the coupling constant, *Jab*= 15–16 Hz indicated that all chalcones obtained were in *trans* configuration. The synthesis of compound **2a-b** is shown in Scheme 2. It involved the formation of acid chloride, followed by esterification to form **2a-b**. The formation of acid chloride was carried out by treating aspirin with oxalyl chloride and N2N-dimethylformamide (as an initiator) in dichloromethane. The synthesized acid chloride intermediates was treated with **1a** and **1b** to generate **2a** and **2b,** respectively (Scheme 2). This method was able to produce complete formation of **2a** and **2b** in 5 h. The IR spectra of **2a-b** showed the disappearance of OH band at 3470-3466 cm^{-1} and the appearance of $\nu_{C=O}$ ester at 1765-1720 cm^{-1}. The absorbance peaks at 2854 cm^{-1} were attributed to the presence of C-H and CH_3. C=O stretch of conjugated keto carbonyl were represented by sharp absorption peak at 1705-1665 cm^{-1}. Furthermore the disappearance of $\nu_{C=O}$ band at 1707 cm^{-1} confirmed the assigned structure of **2a-b.**

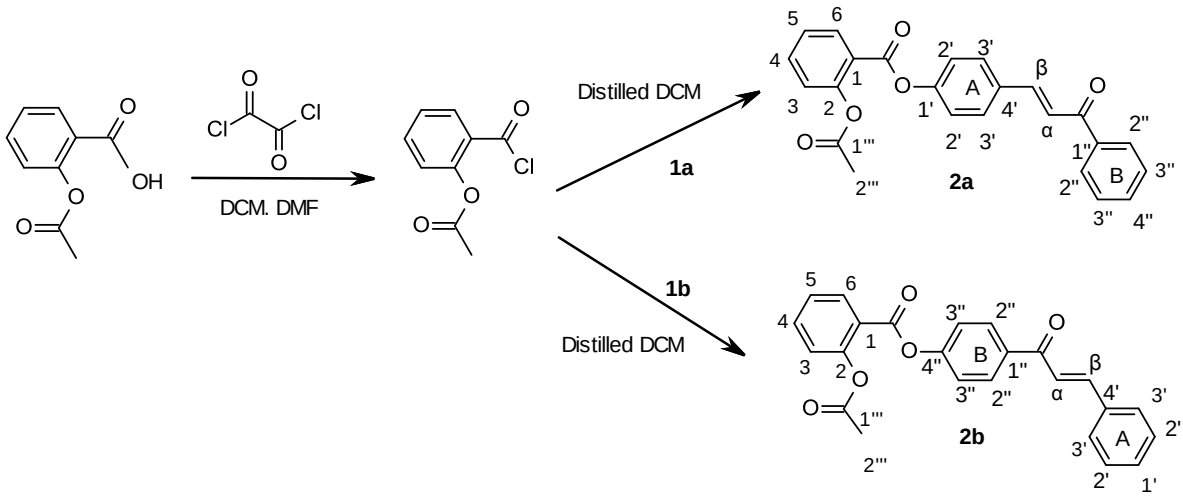
^{1}H NMR spectra of **2a-b** showed the presence of the methyl groups at δ 2.95–3.10. The C=C bonds were represented as two duplets at δ 7.34-8.19 with *Jab*= 15.45 Hz, which indicated *trans* configuration. ^{13}C NMR spectra showed the formation of the C=O of esters group at δ 160.00 -185.00. The C=C of α,β- unsaturated ketone and benzene ring were observed at δ 121.95 -144.24, while the essential two C-O bonds of the compound **2a-b** were observed at δ 150.49-153.98.



1a, R_1 = OH , R_2 = H
1b, R_1 = H , R_2 = OH

Scheme 1. The synthesis of **1a-b**

Antibacterial screening: The antibacterial activities of aspirin and compounds **2a-b** were assayed at the concentration of 50, 80 and 100 ppm against bacteria *E. coli* ATCC 8739 at 37 ^{o}C. The antibacterial activities of **2a-b** were compared with aspirin as a control. The results for antibacterial assay for each compound are shown in Figure 1. All synthesized chalcones demonstrated a similar trend of bacteriostatic activities upon introduction at different concentrations. The equation of $\ln N_t = 27.1 - 8.56T$ was used to indicate the condition of the microbial specific growth and the amount of drug concentration used (Pappano *et al*., 1994). The graph of control (aspirin) and the increasing concentration of the compounds **2a-b** showed no antibacterial activity, which is similar to aspirin when tested against *E. coli*. All the graphs showed that the growth of *E. coli* increase significantly throughout the time. The effect of synthesized chalcones at various concentrations was 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) (Pappano *et al*., 1994). The MIC values for all synthesized compounds were observed to exceed 200 ppm. Compound with the MIC value > 200 ppm is not suitable to be used as antibacterial agent for clinical purposes (Arslan *et al*., 2009).



Scheme 2. The synthesis of **2a-b**

The presence of active functional groups such as C=O and phenyl groups are favor to react with phosphates groups on bacteria surface to inhibit their growth (Zhong *et al*., 2008). However, it was believed that the presence of bulky side chain resulted in various types of effect on antibacterial activities of the aspirin derivatives as they gave the occurrence of steric hindrance which avoids the contact between active sites in the compound with receptor site of the bacteria (Fernandez *et al.*, 2005). Both **2a** and **2b** contained bulky side chain which is built up from the utilized chalcones.

An efficient method to synthesize aspirin-chalcone derivatives **2a-b** was developed by reacting hydroxychalcones **1a-b** with aspirin moieties *via* formation of acid chlorides as intermediates. These newly synthesized aspirin-chalcone derivatives **2a-b** were studied against wild-typed *E. coli* ATCC 8739.

The antibacterial studies of compounds **2a-b** showed poor antibacterial activity with no inhibition against *E. coli.* The attachment of chalcone moiety **1a-b** onto the aspirin scafold was not contributing antibacterial activity.

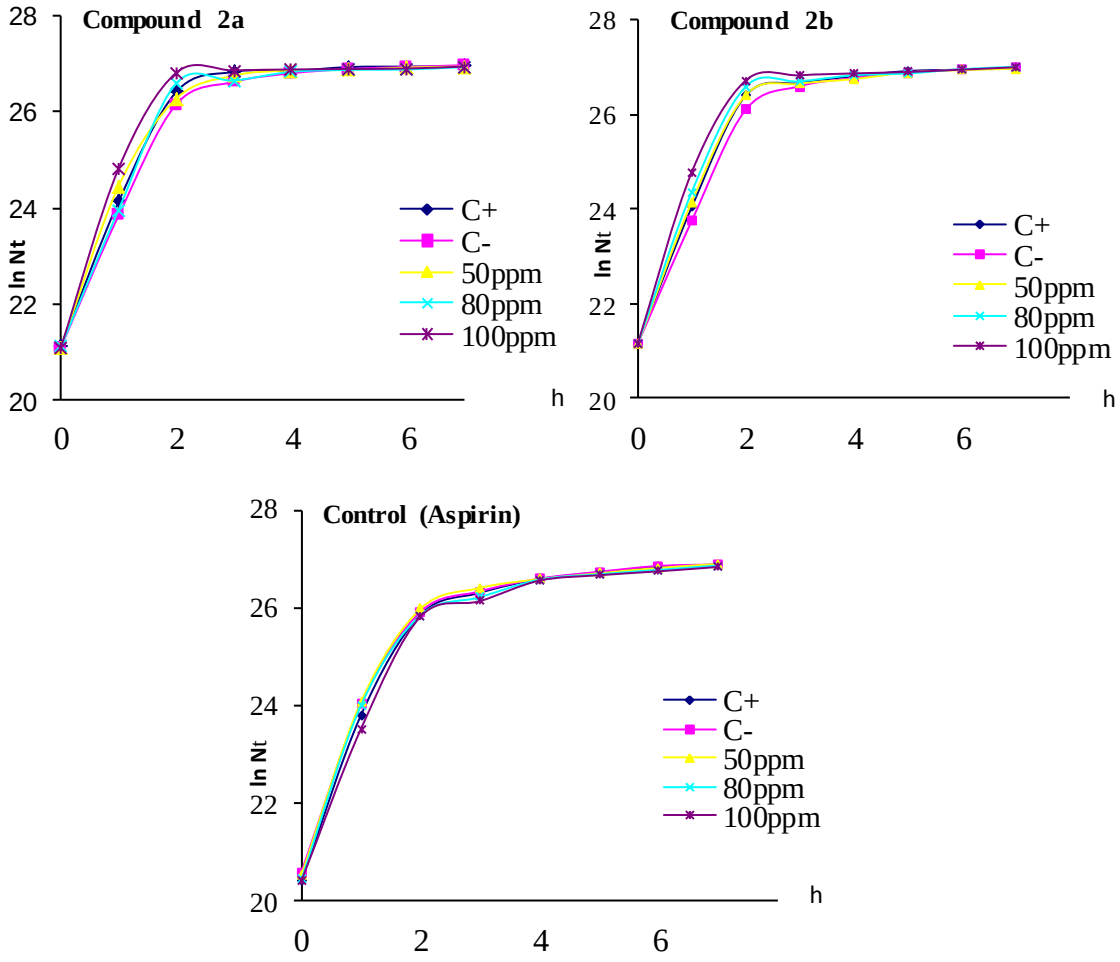


Figure 1. Antibacterial activities of compounds **2a-b** against *E. coli*

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