Biological Studies of Novel Aspirin-Chalcone Derivatives bearing Variable Substituents

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ABSTRACT

The evolution of drug resistant bacteria has now becoming a major concern in the search for new antibacterial agent. Ongoing interest has also developing to find a new class of compounds with antioxidant properties. Herein, a series of hydroxylated chalcones 1a-g and aspirin-chalcone derivatives 2a-g were successfully synthesised for antibacterial and antioxidant properties. Chalcones 1a-g were prepared by Claisen-Schmidt condensation of 4-hydroxyacetophenone and benzaldehyde derivatives, while 2a-g were synthesised via esterification of aspirin with 1a-g. All the synthesised compounds were elucidated using CHNS elemental analysis, FTIR, 1H and 13C NMR spectroscopy, and X-ray crystallography. All compounds were evaluated for antibacterial assay via disc diffusion method and antioxidant assay using stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Only 1a showed moderate activity against *Escherichia coli*, while 1b-g and 2a-g showed no inhibition against *E. coli* and *Staphylococcus aureus* in comparison ampicillin as standard antibiotic. Compounds 1b-g and 2a-g having various substituents contributed to bulky molecular structures and caused difficult penetration into the cell membrane thus, unable to inhibit the bacterial growth. Compounds 1a-g and 2a-g also displayed poor antioxidant properties on DPPH in comparison to ascorbic acid due to low phenolic pharmacophore. The formation of bulky structures for 2a-g have hindered the antioxidant properties compared to 1a-g.

Keywords: Synthesis, chalcone, aspirin, antibacterial activity, antioxidant activity

INTRODUCTION

Aspirin is a well-known non-steroidal anti-inflammatory drug that has been used as medication to treat fever and inflammation for over the century (Vane & Botting, 2003). It has been chemically modified from salicylic acid, an active metabolite which is extracted from bark of Willow tree (Nordin et al., 2018). Prolonged use of aspirin however, can cause adverse effects such as vomiting and stomach bleeding (Vane & Botting, 2003). Structural modification of aspirin has improved its efficacy with less gastrointestinal toxicity compared to...
standard aspirin (Huang et al., 2014). Modification of aspirin by adding various functional groups has displayed wide range of biological properties such as antibacterial, antifungal and anti-inflammatory activities (Nordin et al., 2018).

Chalcone is a compound belongs to flavonoid family and commonly found in fruits, vegetables and other plant products (Panche et al., 2016). Chalcone consists of two phenyl rings and connected by three carbon bridges containing α,β-unsaturated ketone which is claimed to contribute to the activity of chalcone (Al-Rawi et al., 2018). Various substituents was introduced to its molecular structure such as hydroxyl, methoxy or halogen groups which reported for antibacterial, antioxidant, antifungal and many others (Hasan et al., 2015; Kumbhar et al., 2014; Lahsasni et al., 2014). Studies on the properties of chalcones and their biological activities become an interest among researchers, mainly due to its simplicity in synthesis and the versatility of the chalcone structure for chemical modification (Zhuang et al., 2017).

Throughout these years, various bacterial-causing diseases were reported (Rubin & Reisner, 2014). Consequently, antibacterial drugs were manufactured and used to treat these diseases (Gulkok et al., 2012). Improper usage of these drugs however, caused the bacteria to evolve into drug resistant bacteria which reduce the effectiveness of the drugs (Richard-Kortum, 2010). The continuing improvement of new antimicrobial agents is therefore remains a priority (Gulkok et al., 2012).

Apart from bacterial infection, free radical is a reactive intermediate containing unpaired electron which could also cause various diseases (Singhal et al., 2011) and could give adverse effect on lipids, proteins and DNA in human body (Mohana & Kumar, 2013). Antioxidant drug is commonly used to prevent the damages by free radical and inhibit the reactivity (Belsare et al., 2010). Thus, antioxidant drugs were developed to reduce the implications of free radicals (Mohana & Kumar, 2013).

In this paper, hydroxylated chalcones bearing substituents such as methoxy, bromine and chlorine were prepared and incorporated onto aspirin in order to enhance the antibacterial and antioxidant properties possessed by both precursor compounds starting from readily available aspirin. The resulting compound is envisaged to possess greater potential of biological activities. All the synthesized compounds were evaluated against E. coli and S. aureus, and also free radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH).

MATERIALS AND METHODS

Chemical and reagents

4-hydroxyacetophenone, methoxy/chloro/bromobenzaldehyde, potassium hydroxide (KOH), N,N-dimethyl-4-aminopyridine (DMAP) and oxalyl chloride were purchased from Merck. Aspirin and N,N-dicyclohexylcarbodiimide (DCC) were obtained from Acros Organics. Magnesium sulphate (MgSO₄) anhydrous and triethylamine were purchased from J.T. Baker. All other reagents and solvents were used as received without further purification.

Measurements

Melting points of all synthesized compounds were determined on Stuart SMP3 using open tube capillary method and are uncorrected. All compounds were characterised using CHNS Vario MICRO Elementar Analysensysteme GmbH. FTIR spectra were recorded as KBr pellets on Perkin Elmer 1605 FTIR Spectrophotometer. ¹H-NMR and ¹³C-NMR spectra were recorded on JEOL ECA 500 at 500MHz (¹H) and 125MHz (¹³C) with the chemical shift reported relative to DMSO-d₆ as the standard reference and chemical shift values (δ) were expressed in parts per million (ppm). Single crystal X-ray was collected on Bruker APEXII DUO CCD area-detector diffractometer.
General Procedure for the Preparation of Hydroxylated Chalcones 1a-g

Hydroxylated chalcones 1a-g were synthesised via similar method reported by Ngaini et al., (2013) utilising 4-hydroxyacetophenone) and benzaldehyde derivatives.

(E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (1a): Compound 1a was obtained as yellow solid. Yield: 1.04 g (46%), m.p.: 177-178 °C, $\nu_{\text{max}}$ (KBr/cm$^{-1}$) 3136 (O-H), 1646 (C=O), 1606 (Ar-C), 980 (C=CH). $\delta_{\text{H}}$ (500 MHz, DMSO-d$_6$) 6.90 (2H, d, $J$=9.2 Hz, Ar-H), 7.42-7.45 (2H, m, Ar-H), 7.67 (1H, d, $J$=15.3 Hz, C=CH), 7.85-7.86 (3H, m, Ar-H), 7.88 (1H, d, $J$=16.1 Hz, C=CH), 8.07 (2H, d, $J$=8.4 Hz, Ar-H). $\delta_{C}$ (125 MHz, DMSO-d$_6$) 115.4 (Ar-C), 122.1 (C=C), 128.7 (Ar-C), 128.9 (Ar-C), 129.1 (Ar-C), 130.3 (Ar-C), 131.2 (Ar-C), 134.9 (Ar-C), 142.8 (C=C), 162.2 (Ar-C), 187.2 (C=O).

(E)-1-(4-hydroxyphenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (1b): Compound 1b was obtained as yellow solid. Yield: 1.10 g (43%), m.p.: 165-166 °C, $\nu_{\text{max}}$ (KBr/cm$^{-1}$) 3302 (O-H), 2828 (OCH$_3$), 1653 (C=O), 1580 (Ar-C), 974 (C=CH). $\delta_{\text{H}}$ (500 MHz, DMSO-d$_6$) 3.83 (3H, s, OCH$_3$), 6.90 (2H, d, $J$=5.2 Hz, Ar-H), 7.00 (1H, d, $J$=8.6 Hz, Ar-H), 7.33 (1H, t, $J$=8.6 Hz, Ar-H), 7.40 (1H, d, $J$=9.7 Hz, Ar-H), 7.46 (1H, s, Ar-H), 7.64 (1H, d, $J$=14.3 Hz, C=CH), 7.90 (1H, d, $J$=12.1 Hz, C=CH), 8.07 (2H, d, $J$=8.0 Hz, Ar-H). $\delta_{C}$ (125 MHz, DMSO-d$_6$) 55.8 (OCH$_3$), 111.7 (Ar-C), 116.0 (Ar-C), 116.9 (Ar-C), 122.0 (Ar-C), 122.9 (Ar-C), 129.9 (Ar-C), 130.4 (Ar-C), 131.8 (Ar-C), 136.8 (Ar-C), 143.2 (C=C), 160.2 (Ar-C), 162.8 (Ar-C), 187.7 (C=O).

(E)-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (1c): Compound 1c was obtained as yellow solid. Yield: 2.42 g (95%), m.p.: 154-155 °C, $\nu_{\text{max}}$ (KBr/cm$^{-1}$) 3383 (O-H), 2833 (OCH$_3$), 1642 (C=O), 1601 (Ar-C), 1036 (C=CH). $\delta_{\text{H}}$ (500 MHz, DMSO-d$_6$) 3.81 (3H, s, OCH$_3$), 6.89 (2H, d, $J$=8.4 Hz, Ar-H), 6.99 (2H, d, $J$=8.4 Hz, Ar-H), 7.64 (1H, d, $J$=15.3 Hz, C=CH), 7.75 (1H, d, $J$=14.5 Hz, C=CH), 7.80 (2H, d, $J$=10.7 Hz, Ar-H), 8.05 (2H, d, $J$=12.3 Hz, Ar-H). $\delta_{C}$ (125 MHz, DMSO-d$_6$) 55.9 (OCH$_3$), 114.9 (Ar-C), 115.9 (Ar-C), 120.1 (C=C), 128.0 (Ar-C), 129.9 (Ar-C), 131.1 (Ar-C), 131.6 (Ar-C), 143.2 (C=C), 161.6 (Ar-C), 162.6 (Ar-C), 187.6 (C=O).

(E)-3-(2,5-dimethoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (1d): Compound 1d was obtained as yellow crystal. Yield: 1.59 g (56%), m.p.: 167-168 °C, $\nu_{\text{max}}$ (KBr/cm$^{-1}$) 3166 (O-H), 2957 (OCH$_3$), 1643 (C=O), 1591 (Ar-C), 989 (C=CH). $\delta_{\text{H}}$ (500 MHz, DMSO-d$_6$) 3.82 (3H, s, OCH$_3$), 3.88 (3H, s, OCH$_3$), 6.59-6.62 (2H, m, Ar-H), 6.88 (2H, d, $J$=9.2 Hz, Ar-H), 7.69 (1H, d, $J$=16.0 Hz, Ar-H), 7.85 (1H, d, $J$=8.6 Hz, C=CH), 7.91 (1H, d, $J$=16.1 Hz, C=CH), 7.99 (2H, d, $J$=9.2 Hz, Ar-H). $\delta_{C}$ (125 MHz, DMSO-d$_6$) 56.2(OCH$_3$), 56.7 (OCH$_3$), 113.0 (Ar-C), 113.5 (Ar-C), 115.9 (Ar-C), 118.3 (Ar-C), 122.6 (C=C), 124.2 (Ar-C), 129.7 (Ar-C), 131.7 (Ar-C), 137.6 (C=C), 153.1 (Ar-C), 153.8 (Ar-C), 162.7 (Ar-C), 187.8 (C=O).

(E)-3-(3,5-dimethoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (1e): Compound 1e was obtained as yellow solid. Yield: 1.31 g (60%), m.p.: 133-134 °C, $\nu_{\text{max}}$ (KBr/cm$^{-1}$) 3186 (O-H), 2841 (OCH$_3$), 1651 (C=O), 1596 (Ar-C), 974 (C=CH). $\delta_{\text{H}}$ (500 MHz, DMSO-d$_6$) 3.80 (6H, s, OCH$_3$), 6.56 (1H, s, Ar-H), 6.90 (2H, d, $J$=8.4 Hz, Ar-H), 7.05 (2H, s, Ar-H), 7.59 (1H, d, $J$=15.3 Hz, C=CH), 7.90 (1H, d, $J$=15.3 Hz, C=CH), 8.08 (2H, d, $J$=9.2 Hz, Ar-H). $\delta_{C}$ (125 MHz, DMSO-d$_6$) 55.5 (OCH$_3$), 102.6 (Ar-C), 106.6 (Ar-C), 115.4 (Ar-C), 122.6 (C=C), 129.1 (Ar-C), 131.3 (Ar-C), 136.9 (Ar-C), 142.9 (C=C), 160.7 (Ar-C), 162.3 (Ar-C), 187.2 (C=O).

(E)-3-(4-bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (1f): Compound 1f was obtained as yellow solid. Yield: 0.73 g (24%), m.p.: 207-208 °C, $\nu_{\text{max}}$ (KBr/cm$^{-1}$) 3096 (O-H), 1643 (C=O), 1543 (Ar-C), 980 (C=CH). $\delta_{\text{H}}$ (500 MHz, DMSO-d$_6$) 6.90 (2H, d, $J$=8.6 Hz, Ar-H), 7.63-7.66 (3H, m, Ar-H & C=CH), 7.81 (2H, d, $J$=8.6 Hz, Ar-H), 7.93 (1H, d, $J$=15.5 Hz, C=CH), 8.07 (2H, d, $J$=8.6 Hz, Ar-H). $\delta_{C}$ (125 MHz, DMSO-d$_6$) 115.4 (Ar-C), 122.9 (C=C), 123.6 (Ar-C), 129.0 (Ar-C), 130.6 (Ar-C), 131.2 (Ar-C), 131.8 (Ar-C), 134.2 (Ar-C), 141.3 (C=C), 162.3 (Ar-C), 187.0 (C=O).

(E)-3-(4-chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (1g): Compound 1g was obtained as yellow solid. Yield: 1.45 g (56%), m.p.: 228-229 °C, $\nu_{\text{max}}$ (KBr/cm$^{-1}$) 3095 (O-H), 1643 (C=O), 1537 (Ar-C), 981
131.4 (Ar, C=O ester), 188.7 (C=O).

136.0 (Ar), 122.7 (Ar-H & C=CH), 8.07 (2H, d, J=9.2 Hz, Ar-H). δc (125 MHz, DMSO-d6) 115.4 (Ar-C), 122.9 (C=C), 128.8 (Ar-C), 129.0 (Ar-C), 130.3 (Ar-C), 131.2 (Ar-C), 133.8 (Ar-C), 134.7 (Ar-C), 141.2 (C=C), 162.2 (Ar-C), 187.0 (C=O).

### General Procedure for the Preparation of Aspirin-Chalcones Derivatives 2a-g

**Method 1**

Aspirin-chalcones derivatives 2a-g were synthesised using similar method reported by Ngaini et al., (2013) with several modifications. The resulting mixture was filtered and extracted using distilled water (2 x 25 mL). The organic layer was dried over MgSO4 anhydrous and solvent was removed under reduced pressure to form yellow precipitate. The product formed was recrystallized from ethanol to afford 2a-g.

**Method 2**

Aspirin (2 mmol) in 10 mL dry DCM was added to a stirred solution of 1a-g (2 mmol) in 10 mL dry DCM. DCC (2 mmol) and DMAP (1 mmol) in 5 mL dry DCM respectively, were added into the mixture and stirred for 5 min at 0°C. The white precipitate (dicyclohexylurea) formed was filtered off from the reaction. The filtrate was allowed to be stirred at room temperature for 5 h and evaporated under vacuum to form yellow precipitate. The precipitate formed was purified by column chromatography (silica gel, 1:4 ethyl acetate/hexane) to afford 2a-g (Ho et al., 2017).

**E)-4-cinnamoylphenyl 2-acetoxybenzoate (2a):** Compound 2a was obtained as white solid. Yield: 0.26 g, (67%), m.p.: 210-211°C, (Found: C, 74.39; H, 4.89. C12H10O4 Requires C, 74.60; H, 4.70%); \( \nu_{max} \) (KBr/cm\(^{-1}\)) 1766 (C=O ester), 1662 (C-O), 1605 (Ar-C), 1056 (C=CH). δH (500 MHz, DMSO-d6) 2.27 (3H, s, CH3), 7.35 (1H, d, J=8.0 Hz, Ar-H), 7.45-7.48 (4H, m, Ar-H), 7.51 (1H, t, J=8.6 Hz, Ar-H), 7.76-7.82 (2H, m, Ar-H & C=CH), 7.90-7.92 (2H, m, Ar-H), 7.96 (1H, d, J=16.1 Hz, C=CH), 8.20 (2H, d, J=8.0 Hz, Ar-H), 8.28 (2H, d, J=9.8 Hz, Ar-H). δc (125 MHz, DMSO-d6) 20.7 (CH3), 121.9 (Ar-C), 122.2 (C=C), 124.2 (Ar-C), 126.5 (Ar-C), 128.6 (Ar-C), 130.4 (Ar-C), 130.7 (Ar-C), 131.8 (Ar-C), 134.6 (Ar-C), 135.3 (Ar-C), 135.5 (Ar-C), 144.2 (C=C), 150.5 (Ar-C), 153.8 (Ar-C), 162.2 (C=O ester), 169.2 (C=O ester), 188.1 (C=O).

**E)-4-(3-methoxyphenyl)acryloylphenyl 2-acetoxybenzoate (2b):** Compound 2b was obtained as yellowish solid. Yield: 0.13 g (30%), m.p.: 194-195°C, (Found: C, 72.24; H, 5.00. C12H10O4 Requires C, 72.11; H, 4.84%); \( \nu_{max} \) (KBr/cm\(^{-1}\)) 2840 (OCH3), 1767 (C=O ester), 1666 (C=O), 1601 (Ar-C), 1053 (C=CH). δH (500 MHz, DMSO-d6) 2.26 (3H, s, CH3), 3.83 (3H, s, OCH3), 7.03 (1H, d, J=10.7 Hz, Ar-H), 7.34 (1H, d, J=8.4 Hz, Ar-H), 7.38 (1H, d, J=8.4 Hz, Ar-H), 7.45-7.53 (5H, m, Ar-H), 7.74 (1H, d, J=15.3 Hz, C=CH), 7.78 (1H, t, J=8.5 Hz, Ar-H), 7.97 (1H, d, J=16.1 Hz, C=CH), 8.20 (1H, d, J=7.6 Hz, Ar-H), 8.29 (2H, d, J=8.4 Hz, Ar-H). δc (125 MHz, DMSO-d6) 21.3 (CH3), 55.8 (OCH3), 114.0 (Ar-C), 117.3 (C=C), 122.3 (Ar-C), 122.5 (Ar-C), 122.7 (Ar-C), 122.8 (Ar-C), 124.8 (Ar-C), 127.1 (Ar-C), 130.5 (Ar-C), 131.0 (Ar-C), 132.4 (Ar-C), 135.9 (Ar-C), 136.0 (Ar-C), 136.6 (Ar-C), 144.8 (C=C), 151.1 (Ar-C), 154.4 (Ar-C), 160.2 (Ar-C), 162.8 (C=O ester), 169.8 (C=O ester), 188.7 (C=O).

**E)-4-(3-(4-methoxyphenyl)acryloylphenyl 2-acetoxybenzoate (2c):** Compound 2c was obtained as yellowish solid. Yield: 0.19 g (45%), m.p.: 126-128°C, (Found: C, 72.83; H, 5.01. C12H10O4 Requires C, 72.11; H, 4.84%); \( \nu_{max} \) (KBr/cm\(^{-1}\)) 2844 (OCH3), 1769 (C=O ester), 1660 (C=O), 1598 (Ar-C), 1031 (C=CH). δH (500 MHz, DMSO-d6) 2.27 (3H, s, CH3), 3.82 (3H, s, OCH3), 7.02 (2H, d, J=8.4 Hz, Ar-H), 7.34 (1H, d, J=8.4 Hz, Ar-H), 7.43 (2H, d, J=8.4 Hz, Ar-H), 7.50 (1H, t, J=7.7 Hz, Ar-H), 7.74-7.88 (5H, m, Ar-H & C=CH), 8.20 (1H, d, J=6.2 Hz, Ar-H), 8.26 (2H, d, J=8.4 Hz, Ar-H). δc (125 MHz, DMSO-d6) 21.3 (CH3), 55.9 (OCH3), 115.0 (Ar-C), 119.9 (C=C), 122.5 (Ar-C), 122.7 (Ar-C), 124.8 (Ar-C), 127.1 (Ar-C), 127.8 (Ar-C), 130.8 (Ar-C), 131.4 (Ar-C), 132.4 (Ar-C), 135.9 (Ar-C), 136.3 (Ar-C), 144.8 (C=C), 151.1 (Ar-C), 154.2 (Ar-C), 162.0 (Ar-C), 162.8 (C=O ester), 169.8 (C=O ester), 188.5 (C=O).
in DMSO was placed on the surface of MHA plate using a sterile pair of forceps. The plates were then incubated.

Cotton suspension prepared was inoculated onto the entire surface of a Mueller-)

The antibacterial activities of the synthesized compounds were evaluated against E. coli ATCC 25922 and S. aureus S48/81 using disc diffusion method. E. coli and S. aureus were used as inoculum where it was cultured in Mueller-Hinton Broth (MHB) and incubated at 37 °C with permanent shaking at 180 rpm for 18 h. The bacterial suspension prepared was inoculated onto the entire surface of a Mueller-Hinton Agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. Sterile filter paper disc impregnated with 10 μL of the compound in DMSO was placed on the surface of MHA plate using a sterile pair of forceps. The plates were then incubated at 37 °C for 24 h. Ampicillin was used as a positive control, DMSO as a negative control and aspirin as a

**Antibacterial Assay**

The antibacterial activities of the synthesized compounds were evaluated against E. coli ATCC 25922 and S. aureus S48/81 using disc diffusion method. E. coli and S. aureus were used as inoculum where it was cultured in Mueller-Hinton Broth (MHB) and incubated at 37 °C with permanent shaking at 180 rpm for 18 h. The bacterial suspension prepared was inoculated onto the entire surface of a Mueller-Hinton Agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. Sterile filter paper disc impregnated with 10 μL of the compound in DMSO was placed on the surface of MHA plate using a sterile pair of forceps. The plates were then incubated at 37 °C for 24 h. Ampicillin was used as a positive control, DMSO as a negative control and aspirin as a

(E)-4-(3-(2,5-dimethoxyphenyl)acryloyl)phenyl 2-acetoxybenzoate (2d): Compound 2d was obtained as yellow solid. Yield: 0.09 g (20%), m.p.: 121-122 °C, (Found: C, 69.27; H, 5.09. C$_{28}$H$_{36}$O$_{6}$S: Requires C, 69.95; H, 4.97%); $\delta_{\text{max}}$ (KBr/cm$^{-1}$) 3020 (O=C), 2920 (OCH$_3$), 1714 (C=O ester), 1691 (C=O), 1598 (Ar-C), 1011 (C=C-H). δ(H, δ(C=O), 188.6 (C=O).

(E)-4-(3-(3,5-dimethoxyphenyl)acryloyl)phenyl 2-acetoxybenzoate (2e): Compound 2e was obtained as yellowish solid. Yield: 0.36 g (40%), m.p.: 124-125 °C, (Found: C, 69.47; H, 4.87. C$_{28}$H$_{36}$O$_{6}$S: Requires C, 69.95; H, 4.97%); $\delta_{\text{max}}$ (KBr/cm$^{-1}$) 2945 (OCH$_3$), 1767 (C=O ester), 1664 (C=O), 1605 (Ar-C), 1049 (C=CH-H). δ(H, δ(C=O), 162.8 (C=O ester), 169.8 (C=O ester), 188.7 (C=O).

(E)-4-(3-(3,5-dimethoxyphenyl)acryloyl)phenyl 2-acetoxybenzoate (2f): Compound 2f was obtained as white solid. Yield: 0.12 g (26%), m.p.: 251-252 °C, (Found: C, 61.36; H, 3.94. C$_{28}$H$_{36}$O$_{6}$S: Requires C, 61.95; H, 3.68%); $\delta_{\text{max}}$ (KBr/cm$^{-1}$) 1745 (C=O ester), 1658 (C=O), 1603 (Ar-C), 1047 (C=CH-H). δ(H, δ(C=O), 162.8 (C=O ester), 169.8 (C=O ester), 188.7 (C=O).

(E)-4-(3-(4-bromophenyl)acryloyl)phenyl 2-acetoxybenzoate (2g): Compound 2g was obtained as white solid. Yield: 0.22 g (26%), m.p.: 228-230 °C, (Found: C, 68.28; H, 3.69. C$_{28}$H$_{36}$O$_{6}$S: Requires C, 68.50; H, 4.07%); $\delta_{\text{max}}$ (KBr/cm$^{-1}$) 1746 (C=O ester), 1675 (C=O), 1600 (Ar-C), 1038 (C=CH-H). δ(H, δ(C=O), 162.8 (C=O ester), 169.8 (C=O ester), 188.0 (C=O).

(E)-4-(3-(4-chlorophenyl)acryloyl)phenyl 2-acetoxybenzoate (2h): Compound 2h was obtained as white solid. Yield: 0.22 g (26%), m.p.: 228-230 °C, (Found: C, 68.28; H, 3.69. C$_{28}$H$_{36}$O$_{6}$S: Requires C, 68.50; H, 4.07%); $\delta_{\text{max}}$ (KBr/cm$^{-1}$) 1746 (C=O ester), 1675 (C=O), 1600 (Ar-C), 1038 (C=CH-H). δ(H, δ(C=O), 162.8 (C=O ester), 169.8 (C=O ester), 188.0 (C=O).

(E)-4-(3-(2,5-dimethoxyphenyl)acryloyl)phenyl 2-acetoxybenzoate (2d): Compound 2d was obtained as yellow solid. Yield: 0.09 g (20%), m.p.: 121-122 °C, (Found: C, 69.27; H, 5.09. C$_{28}$H$_{36}$O$_{6}$S: Requires C, 69.95; H, 4.97%); $\delta_{\text{max}}$ (KBr/cm$^{-1}$) 2920 (OCH$_3$), 1714 (C=O ester), 1691 (C=O), 1598 (Ar-C), 1011 (C=C-H). δ(H, δ(C=O), 188.6 (C=O).
The zones of inhibition were measured in millimeter (mm) to estimate the potency of the test compounds (Sie et al., 2018).

**Antioxidant Assay**

The free radical scavenging activities of the synthesized compounds were determined using stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH solution in methanol was prepared with resulting concentration of 0.1 mM. Increasing concentrations of tested compounds and aspirin (6.25, 12.50, 50.00, 100.00 and 200.00 ppm) were prepared using serial dilution in methanol. The tested compound solution (1 mL) was mixed with 4 mL of DPPH solution and 1 mL of methanol was mixed with 4 mL of DPPH solution as blank. The mixtures were kept from light for 30 min and the absorbance of tested solutions was recorded on Optima SP-300 spectrophotometer at 517 nm. Ascorbic acid was used as standard reference. The IC<sub>50</sub> values for all compounds were determined by plotting the scavenging activity versus concentration graph (Murti et al., 2013).

**RESULTS AND DISCUSSION**

**Chemistry**

Hydroxylated chalcones 1a-g were synthesized in base-catalyzed reaction via Claisen-Schmidt condensation of 4-hydroxyacetophenone and benzaldehyde derivatives to form yellow solid (24-95%) (Fig. 1) (Ngaini et al., 2012b). The low yield obtained could be due to formation of Cannizzaro side reaction or ketone auto condensation (Ngaini et al., 2012b).

![Fig. 1. Synthesis of hydroxylated chalcones 1a-g](image)

The FTIR spectra of 1a-g showed broad absorption peaks at 3423-3095 cm<sup>-1</sup> attributed to –OH groups. The presence of methoxy group in the chalcones was indicated by peaks at 2957-2828 cm<sup>-1</sup>. The frequency at 1653-1634 cm<sup>-1</sup> was corresponded to C=O, while the frequency at 1608-1537 cm<sup>-1</sup> were attributed to aromatic groups. The frequency at 1036-974 cm<sup>-1</sup> resulting from the effects of conjugation with C=O in the molecular structure (Williams & Fleming, 1995).

The <sup>1</sup>H NMR spectra indicated significant resonance for methoxy group which observed at 3.80-3.88 ppm as a singlet. The aromatic groups were represented by a multiplet in the range of 6.56-8.21 ppm. The formation of chalcones were confirmed by resonances at 7.59-7.94 ppm for C=CH with two doublets (Lahsasni et al., 2014). Broad peaks at 10.41-10.52 ppm were assigned for hydroxyl group where the signals were at lower field due to several factors such as the effects of hydrogen bond, temperature and solvent (Williams & Fleming, 1995). For <sup>13</sup>C NMR, the presence of methoxy groups were confirmed by peaks at 55.5-56.7 ppm, while resonances in the range of 98.8-163.4 ppm were attributed to the aromatic groups. The formation of chalcones was also supported
by the peaks at 106.8-143.2 ppm which corresponded to vinylic carbons. The presence of carbonyl group was observed at 187.0-187.9 ppm.

Only 1d was successfully produced single crystal for analysis. X-ray crystallography study confirmed the formation of 1d with the hydroxyl group at para position in ring A and 2,5-OCH$_3$ group at ring B (Fig. 2).

Fig. 2. X-ray crystallography of 1d

Compounds 1a-g were then incorporated onto aspirin by esterification reaction to form aspirin-chalcone derivatives 2a-g (Fig. 3).

Fig. 3. Proposed synthesis of aspirin-chalcone derivatives 2a-g

Compounds 2a-g were synthesized by reacting hydroxylated chalcones 1a-g with acetylsalicyloyl chloride from the reaction of aspirin with oxalyl chloride in the presence of DMF as initiator with yield range 13-20% (Fig. 4) (Ngaini et al., 2012a). The low yield obtained is due to the presence of electron withdrawing group such as bromine and chlorine which decreased the nucleophilic properties of chalcones during reaction (Belharouak & Pol, 2012).

Fig. 4. Synthesis of 2a-g utilizing Et$_3$N
Higher yield was obtained using dicyclohexylcarbodiimide (DCC) as a stronger coupling agent (Lele et al., 1999) and dimethylaminopyridine (DMAP) as catalyst (Tsvetkova et al., 2006) with 20-67% (Fig. 5).

![Fig. 5. Synthesis of 2a-g utilizing DCC/DMAP](image)

The FTIR spectra supported the formation of aspirin-chalcone derivatives 2a-g by disappearance of broad peak attributed to the hydroxyl group in hydroxylated chalcones 1a-g. The appearance of sharp peaks at 1769-1734 cm⁻¹ was corresponded to C=O ester groups (Motan & Pui, 2014). Peaks at 1605-1600 cm⁻¹ were attributed to aromatic groups, whereas the C=C in the chalcone moiety were represented by absorption bands at 1056-1038 cm⁻¹ (Williams & Fleming, 1995).

The ¹H NMR spectra for 2a-g showed –CH₃ of aspirin at 2.26-2.27 ppm, while singlets at 3.81-3.82 ppm attributed to methoxy group. Multiplet resonances at 6.60-8.28 ppm were corresponded to aromatic groups, whereas the C=CH group were represented by peaks at 7.68-8.01 ppm. In ¹³C NMR spectra, the resonances at 20.7-21.3 and 55.9-56.0 ppm were assigned for methyl and methoxy groups, respectively. The peaks in the range of 103.5-162.8 ppm were attributed to aromatic carbons, whereas the peaks for C=C were observed at 119.9-148.8 ppm. The presence of two ester groups confirmed by the two peaks corresponded to C=O(ester) at 162.2 and 169.8 ppm. The carbonyl groups in the chalcone moieties were represented by peaks at 188.1-188.7 ppm.

**Antibacterial Activities**

The inhibition of *E. coli* and *S. aureus* were measured via diameter of inhibition zone for 1a-g and 2a-g in comparison to the inhibition zone of ampicillin as shown in **Table 1**.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Zone of Inhibition (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>1a</td>
<td>8.0</td>
</tr>
<tr>
<td>1b</td>
<td>-</td>
</tr>
<tr>
<td>1c</td>
<td>-</td>
</tr>
<tr>
<td>1d</td>
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<td>1e</td>
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<td>1g</td>
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<td>2a</td>
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<td>2c</td>
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<td>-</td>
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<tr>
<td>2g</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>16.0</td>
</tr>
</tbody>
</table>

27
The results showed that only 1a exhibited moderate inhibition towards growth of *E. coli* and no inhibition against *S. aureus*. The difference in cell membrane component of *S. aureus* with thicker cell wall could be the cause of ineffectiveness in cell membrane penetration compared to *E. coli* (Lopez-Romero *et al.*, 2015), while 1b-g showed no inhibition against both *E. coli* and *S. aureus*. The presence of methoxy, bromine and chlorine substituents contributed to bulky structure and caused difficult penetration into the cell membrane of bacteria (Ngaini & Ho 2017). No inhibition by 2a-g could be due to the additional bulky structures in the compounds which hindered the penetration of tested compounds into the cell wall of bacteria.

**Antioxidant Activity**

The scavenging activities of all the compounds are depicted in Fig. 6 and Fig. 7. Compounds 2e-f were not tested due to poor solubility in methanol.

![Fig. 6. Free radical scavenging activity of 1a-g](image)

![Fig. 7. Free radical scavenging activity of 2a-g](image)

The graph showed that all tested compounds are very weak free radical scavenger with IC$_{50}$ of more than 200 ppm compared to ascorbic acid, IC$_{50}$ = 12.2 ppm. The addition of aspirin onto chalcones did not enhance the antioxidant activity. Poor performance of 1a-g as antioxidant could be due to low phenolic pharmacophore presence in the molecules (Jin *et al.*, 2012). Compound 1a showed slight activity compared to 1b-g due to smaller
molecular size, while 2a-g have larger molecular structure which unable to combine with DPPH radical caused by the steric hindrance (Weng & Huang, 2014). The incorporation of chalcones onto aspirin resulted in bulky molecular structures with no biological properties.

CONCLUSION

A series of aspirin chalcone derivatives 2a-g were successfully synthesized by incorporation of aspirin and hydroxylated chalcones 1a-g via esterification. All synthesized compounds displayed weak antibacterial activities against E. coli and S. aureus, and poor scavenging activity on DPPH. The incorporation of chalcones onto aspirin resulted in bulky compounds which prevent interaction between receptors, thus reduced the biological activities.

ABBREVIATIONS

CHNS: Carbon, Hydrogen, Nitrogen & Sulfur; DCC: N,N-dicyclohexylcarbodiimide; DCM: Dichloromethane; DMAP: N,N-dimethyl-4-aminopyridine; DMF: Dimethylformamide; DMSO: Dimethylsulfoxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FTIR: Fourier Transform Infrared; HCl: Hydrochloric acid; IC_{50}: Half-maximal inhibitory concentration; KOH: potassium hydroxide; MgSO_4: Magnesium sulphate; MHA: Mueller-Hinton Agar; MHB: Mueller-Hinton Broth; NMR: Nuclear Magnetic Resonance.

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