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# 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, $^{1}H$ and $^{13}C$ 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: S**ynthesis, 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 ($MgSO_4$) 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. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on JEOL ECA 500 at 500MHz ($^{1}$H) and 125MHz ($^{13}$C) with the chemical shift reported relative to DMSO-$d_6$ 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, $\nu_{max}$ (KBr/cm$^{-1}$) 3136 (O-H), 1646 (C=O), 1606 (Ar-C), 980 (C=CH). $\delta_H$ (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, $\nu_{max}$ (KBr/cm$^{-1}$) 3302 (O-H), 2828 ($OCH_3$), 1653 (C=O), 1580 (Ar-C), 974 (C=CH). $\delta_H$ (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 (C=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, $\nu_{max}$ (KBr/cm$^{-1}$) 3383 (O-H), 2833 ($OCH_3$), 1642 (C=O), 1601 (Ar-C), 1036 (C=CH). $\delta_H$ (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, $\nu_{max}$ (KBr/cm$^{-1}$) 3166 (O-H), 2957 ($OCH_3$), 1643 (C=O), 1591 (Ar-C), 989 (C=CH). $\delta_H$ (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, $\nu_{max}$ (KBr/cm$^{-1}$) 3186 (O-H), 2841 ($OCH_3$), 1651 (C=O), 1596 (Ar-C), 974 (C=CH). $\delta_H$ (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), (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, $\nu_{max}$ (KBr/ cm$^{-1}$) 3096 (O-H), 1643 (C=O), 1543 (Ar-C), 980 (C=CH). $\delta_H$ (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, $\nu_{max}$ (KBr/cm$^{-1}$) 3095 (O-H), 1643 (C=O), 1537 (Ar-C), 981

(C=CH). $\delta_H$ (500 MHz, DMSO-$d_6$) 6.90 (2H, d, *J*=8.6 Hz, Ar-H), 7.49 (2H, d, *J*=8.6 Hz, Ar-H), 7.65 (1H, d, *J*=15.5 Hz, C=CH), 7.90-7.94 (3H, m, Ar-H & C=CH), 8.07 (2H, d, *J*=9.2 Hz, Ar-H). $\delta_C$ (125 MHz, DMSO-$d_6$) 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 $MgSO_4$ 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. $C_{24}H_{18}O_5$ 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). $\delta_H$ (500 MHz, DMSO-$d_6$) 2.27 (3H, s, $CH_3$), 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). $\delta_C$ (125 MHz, DMSO-$d_6$) 20.7 ($CH_3$), 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-(3-methoxyphenyl)acryloyl)phenyl 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. $C_{25}H_{20}O_6$ Requires C, 72.11; H, 4.84%); $\nu_{max}$ (KBr/cm$^{-1}$) 2840 ($OCH_3$), 1767 (C=O ester), 1666 (C=O), 1601 (Ar-C), 1053 (C=CH). $\delta_H$ (500 MHz, DMSO-$d_6$) 2.26 (3H, s, $CH_3$), 3.83 (3H, s, $OCH_3$), 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). $\delta_C$ (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 55.8 ($OCH_3$), 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)acryloyl)phenyl 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. $C_{25}H_{20}O_6$ Requires C, 72.11; H, 4.84%); $\nu_{max}$ (KBr/cm$^{-1}$) 2844 ($OCH_3$), 1769 (C=O ester), 1660 (C=O), 1598 (Ar-C), 1031 (C=CH). $\delta_H$ (500 MHz, DMSO-$d_6$) 2.27 (3H, s, $CH_3$), 3.82 (3H, s, $OCH_3$), 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). $\delta_C$ (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 55.9 ($OCH_3$), 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).

**(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. $C_{26}H_{22}O_7$ Requires C, 69.95; H, 4.97%); $\nu_{max}$ (KBr/cm$^{-1}$) 2920 ($OCH_3$), 1741 (C=O ester), 1691 (C=O), 1598 (Ar-C), 1011 (C=CH). $\delta_H$ (500 MHz, DMSO-$d_6$) 2.26 (3H, s, $CH_3$), 3.80 (3H, s, $OCH_3$), 3.85 (3H, s, $OCH_3$), 7.06 (2H, d, Ar-H), 7.35 (1H, d, *J*=6.9 Hz, Ar-H), 7.43 (2H, d, *J*=8.5 Hz, Ar-H), 7.51 (1H, t, *J*=7.6 Hz, Ar-H), 7.58 (1H, s, Ar-H), 7.79 (1H, t, *J*=6.2 Hz, Ar-H), 7.92 (1H, d, *J*=16.1 Hz, C=CH), 8.05 (1H, d, *J*=15.3 Hz, C=CH), 8.20 (1H, d, *J*=6.1 Hz, Ar-H), 8.26 (2H, d, *J*=8.5 Hz, Ar-H). $\delta_C$ (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 56.2 ($OCH_3$), 56.7 ($OCH_3$), 113.1 (Ar-C), 113.6 (Ar-C), 118.9 (Ar-C), 122.4 (Ar-C), 122.5 (Ar-C), 122.8 (Ar-C), 123.9 (Ar-C), 124.8 (Ar-C), 127.1 (C=C), 130.9 (Ar-C), 132.4 (Ar-C), 135.9 (Ar-C), 136.2 (Ar-C), 139.0 (C=C), 151.0 (Ar-C), 153.3 (Ar-C), 153.8 (Ar-C), 154.3 (Ar-C), 162.8 (C=O ester), 169.8 (C=O ester), 188.7 (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. $C_{26}H_{22}O_7$ Requires C, 69.95; H, 4.97%); $\nu_{max}$ (KBr/cm$^{-1}$) 2945 ($OCH_3$), 1767 (C=O ester), 1664 (C=O), 1605 (Ar-C), 1049 (C=CH). $\delta_H$ (500 MHz, DMSO-$d_6$) 2.26 (3H, s, $CH_3$), 3.81 (6H, s, $OCH_3$), 6.60 (1H, s, Ar-H), 7.09 (2H, s, Ar-H), 7.34 (1H, d, *J*=9.2 Hz, Ar-H), 7.44 (2H, d, *J*=8.6 Hz, Ar-H), 7.50 (1H, t, *J*=8.0 Hz, Ar-H), 7.68 (1H, d, *J*=15.5 Hz, C=CH), 7.78 (1H, t, *J*=9.2 Hz, Ar-H), 7.95 (1H, d, *J*=16.1 Hz, C=CH), 8.19 (1H, d, *J*=10.9 Hz, Ar-H), 8.28 (2H, d, *J*=10.9 Hz, Ar-H). $\delta_C$ (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 56.0 ($OCH_3$), 103.5 (Ar-C), 107.3 (Ar-C), 122.5 (Ar-C), 122.8 (Ar-C), 123.0 (Ar-C), 124.8 (Ar-C), 127.1 (C=C), 131.0 (Ar-C), 132.4 (Ar-C), 135.9 (Ar-C), 136.0 (Ar-C), 137.1 (Ar-C), 144.9 (C=C), 151.0 (Ar-C), 154.4 (Ar-C), 161.3 (Ar-C), 162.8 (C=O ester), 169.8 (C=O ester), 188.7 (C=O).

**(E)-4-(3-(4-bromophenyl)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. $C_{24}H_{17}BrO_6$ Requires C, 61.95; H, 3.68%); $\nu_{max}$ (KBr/cm$^{-1}$) 1745 (C=O ester), 1658 (C=O), 1603 (Ar-C), 1047 (C=CH). $\delta_H$ (500 MHz, DMSO-$d_6$) 2.26 (3H, s, $CH_3$), 7.35 (1H, d, *J*=8.6 Hz, Ar-H), 7.45 (2H, d, *J*=8.6 Hz, Ar-H), 7.51 (1H, t, *J*=7.5 Hz, Ar-H), 7.67 (2H, d, *J*=8.6 Hz, Ar-H), 7.73 (1H, d, *J*=16.0 Hz, C=CH), 7.79 (1H, t, *J*=7.4 Hz, Ar-H), 7.88 (2H, d, *J*=8.6 Hz, Ar-H), 8.01 (1H, d, *J*=15.5 Hz, C=CH), 8.20 (1H, d, *J*=8.6 Hz, Ar-H), 8.28 (2H, d, *J*=8.6 Hz, Ar-H). $\delta_C$ (125 MHz, DMSO-$d_6$) 20.7 ($CH_3$), 121.9 (Ar-C), 122.2 (C=C), 122.7 (Ar-C), 124.0 (Ar-C), 124.2 (Ar-C), 126.5 (Ar-C), 130.4 (Ar-C), 130.8 (Ar-C), 131.9 (Ar-C), 133.9 (Ar-C), 135.3 (Ar-C), 135.4 (Ar-C), 142.8 (C=C), 150.5 (Ar-C), 153.9 (Ar-C), 162.2 (C=O), 169.2 (C=O ester), 188.0 (C=O).

**(E)-4-(3-(4-chlorophenyl)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. $C_{25}H_{20}O_6$ Requires C, 68.50; H, 4.07%); $\nu_{max}$ (KBr/cm$^{-1}$) 1746 (C=O ester), 1675 (C=O), 1600 (Ar-C), 1038 (C=CH). $\delta_H$ (500 MHz, DMSO-$d_6$) 2.26 (3H, s, $CH_3$), 7.34 (1H, d, *J*=8.4 Hz, Ar-H), 7.44 (2H, d, *J*=8.4 Hz, Ar-H), 7.52-7.54 (3H, m, Ar-H), 7.74 (1H, d, *J*=16.1 Hz, C=CH), 7.79 (1H, t, *J*=7.7 Hz, Ar-H), 7.93 (2H, d, *J*=8.4 Hz, Ar-H), 7.97 (1H, d, *J*=15.3 Hz, C=CH), 8.19 (1H, d, *J*=8.5 Hz, Ar-H), 8.27 (2H, d, *J*=8.4 Hz, Ar-H). $\delta_C$ (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 122.4 (Ar-C), 122.8 (C=C), 123.2 (Ar-C), 124.8 (Ar-C), 127.1 (Ar-C), 129.5 (Ar-C), 131.0 (Ar-C), 131.2 (Ar-C), 132.4 (Ar-C), 134.1 (Ar-C), 135.7 (Ar-C), 135.9 (Ar-C), 143.3 (C=C), 151.0 (Ar-C), 154.4 (Ar-C), 162.8 (C=O ester), 169.8 (C=O ester), 188.6 (C=O).

**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. Steriled 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

reference. 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 $IC_{50}$ 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).

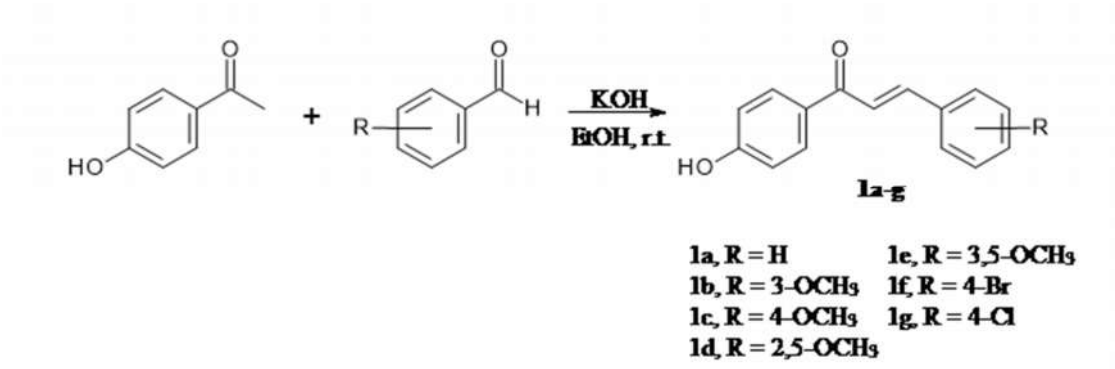


**Fig. 1.** Synthesis of hydroxylated chalcones **1a-g**

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

The $^{1}H$ 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 $^{13}C$ 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**).

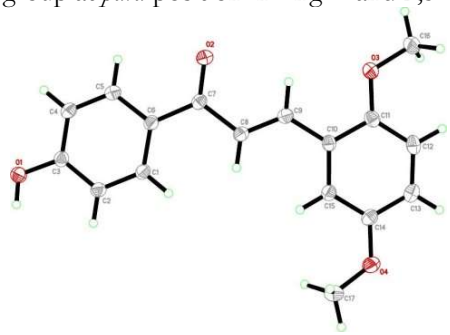

**Fig. 2.** X-ray crystallography of **1d**

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

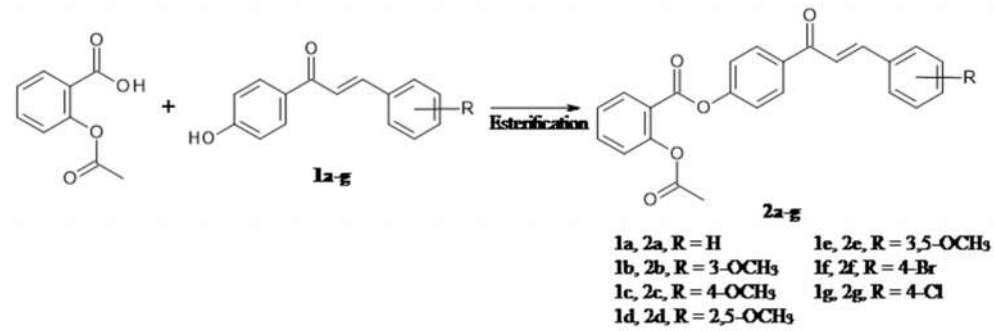


**Fig. 3.** Proposed synthesis of aspirin-chalcone derivatives **2a-g**

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).

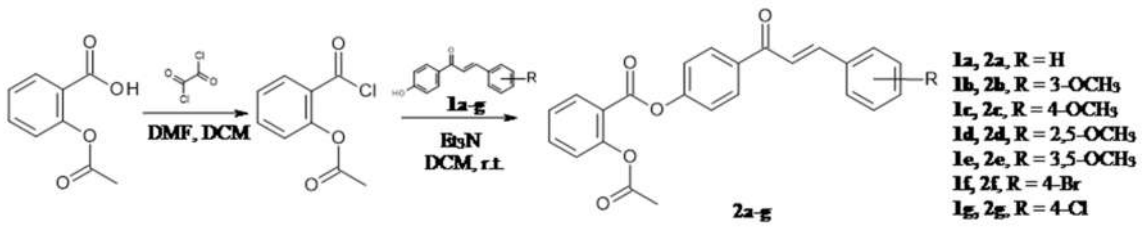


**Fig. 4.** Synthesis of **2a-g** utilizing $Et_3N$

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**).

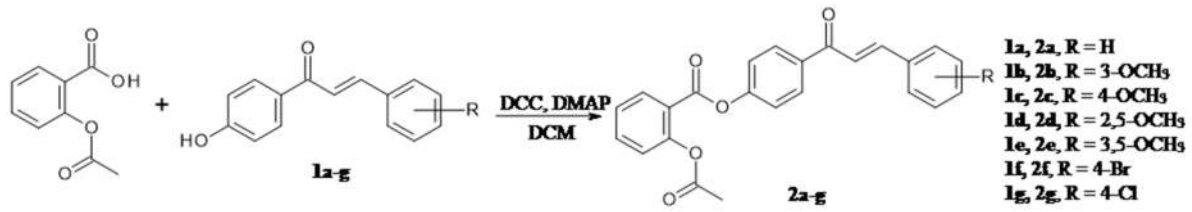


**Fig. 5.** Synthesis of **2a-g** utilizing DCC/DMAP

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 $^{1}H$ NMR spectra for **2a-g** showed $–CH_3$ 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 $^{13}C$ 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 1.** Inhibition zones of aspirin, ampicillin, **1a-g** and **2a-g**

| Compounds | Zone of Inhibition (mm) | |
|---|---|---|
| | *E. coli* | *S. aureus* |
| **1a** | 8.0 | - |
| **1b** | - | - |
| **1c** | - | - |
| **1d** | - | - |
| **1e** | - | - |
| **1f** | - | - |
| **1g** | - | - |
| **2a** | - | - |
| **2b** | - | - |
| **2c** | - | - |
| **2d** | - | - |
| **2e** | - | - |
| **2f** | - | - |
| **2g** | - | - |
| Aspirin | - | - |
| DMSO | - | - |
| Ampicillin | 16.0 | 11.0 |

Note: (-) = No activity

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.

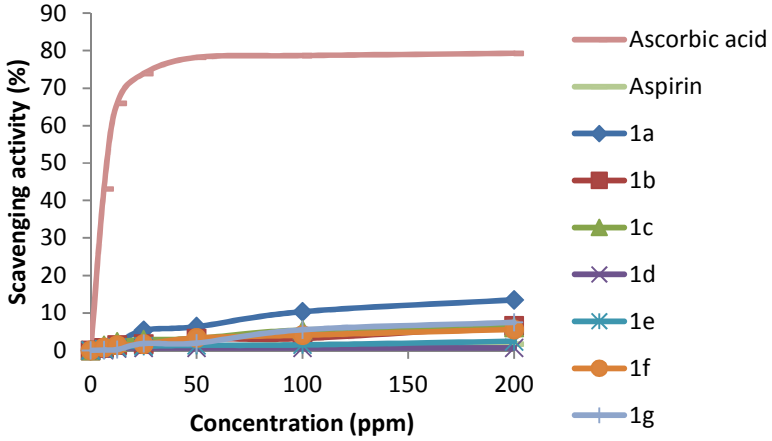


**Fig. 6.** Free radical scavenging activity of **1a-g**

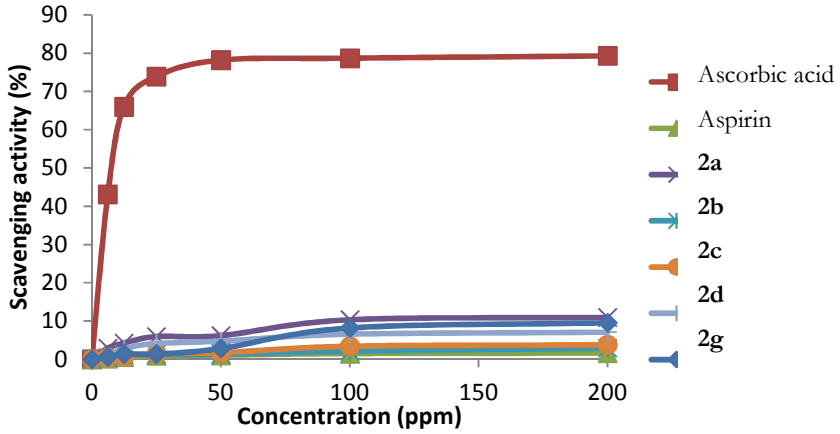


**Fig. 7.** Free radical scavenging activity of **2a-g**

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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## REFERENCES

Al-Rawi, M. S., Hassan, H. A., Hassan, D. F., & Majeed, I. Y. (2018). New series of substituted heterocyclics derived from α, β–unsaturated ketone and their cytotoxic activity tumor cell lines. *Oriental Journal of Chemistry*, *34*(6), 2826-2831.

Belharouak, I., & Pol, V. G. (2012). *Advances in inorganic phosphate materials: Ceramic transactions*. United States of America: John Wiley & Sons.

Belsare, D.P., Pal, S.C., Kazi, A.A., Kankate, R.S., & Vanjari, S.S. (2010). Evaluation of antioxidant activity of chalcones and flavonoids. *International Journal of ChemTech Research, 2*(2), 1080-1089.

Gulkok, Y., Bicer, T., Onurdag, F.K., Ozgen, S., Sahin, M.F., & Dogruer, D.S. (2012). Synthesis of some new thiourea derivatives and evaluation of their antimicrobial activities. *Turkish Journal of Chemistry, 36*, 279-291.

Hasan, S. A., Elias, A. N., & Farhan, M. S. (2015). Synthesis, characterization and antimicrobial evaluation of a series of chalcone derivatives. *Der Pharma Chemica, 7*(2), 39-42.

Ho, B. K., Ngaini, Z., Matthew Neilsen, P., Hwang, S. S., Entigu Linton, R., Kong, E. L., & Lee, B. K. (2017). Synthesis and anticancer activities of 4-[(halophenyl) diazenyl] phenol and 4-[(halophenyl) diazenyl] phenyl aspirinate derivatives against nasopharyngeal cancer cell lines. *Journal of Chemistry, 2017*, 1-7.

Huang, L., Wong, C. C., Mackenzie, G. G., Sun, Y., Cheng, K. W., Vrankova, K., Alston, N., Ouyang, N., & Rigas, B. (2014). Phospho-aspirin (MDC-22) inhibits breast cancer in preclinical animal models: An effect mediated by EGFR inhibition, p53 acetylation and oxidative stress. *BMC Cancer, 14*(141), 1-14.

Jin, L., Zhang, Y., Yan, L., Guo, Y., & Niu, L. (2012). Phenolic compounds and antioxidant activity of bulb extracts of six *Lilium* species native to China. *Molecules, 17*, 9361-9378.

Kumbhar, D. D., Waghamare, B. Y., Pathade, G. R., & Pardeshi, S. K. (2014). Synthesis and evaluation of chalcones as an antibacterial and antifungal agents. *Der Pharmacia Lettre, 6*(1), 224-229.

Lahsasni, S. A., Korni, F. H. A., & Aljaber, N. A. (2014). Synthesis, characterization and evaluation of antioxidant activities of some novel chalcones analogues. *Chemistry Central Journal, 8*(32), 1-10.

Lele, B. S., Gore, M. A., & Kulkarni, M. G. (1999). Direct esterification of poly(ethylene glycol) with amino acid hydrochlorides. *Synthetic Communications, 29*(10), 1727-1739.

Lopez-Romero, J. C., Gonzalez-Rios, H., Borges, A., & Simoes, M. (2015). Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. *Evidence-Based Complementary and Alternative Medicine, 2015*, DOI: 10.1155/2015/795435

Mohana, K.N., & Kumar, C.B.P. (2013). Synthesis and antioxidant activity of 2-amino-5-methylthiazol derivatives containing 1,3,4-oxadiazole-2-thiol moiety. *ISRN Organic Chemistry, 2013*, 1-8.

Motan, G., & Pui, A. (2014). Studies of different types of aspirin by spectrophotometric methods. *Acta Chemica Iasi, 22*(2), 155-164.

Murti, Y., Goswami, A., & Mishra, P. (2013). Synthesis and antioxidant activity of some chalcones and flavonoids. *International Journal of PharmTech Research, 5*(2), 811-818.Ngaini, Z., & Ho, B. K. (2017). Synthesis and antibacterial activity of azo and aspirin-azo derivatives. *Malaysian Journal of Analytical Sciences*, *21*(5), 1183-1194.

Ngaini, Z., Arif, M. A. M., Hussain, H., Er, S. M., Tang, D., & Kamaluddin, D. H. A. (2012a). Synthesis and antibacterial activity of acetoxybenzoyl thioureas with aryl and amino acid side chains. *Phosphorus, Sulfur, and Silicon and the Related Elements, 187*(1), 1-7.

Ngaini, Z., Haris-Fadzillah, S. M., & Hussain, H. (2012b). Synthesis and antimicrobial studies of hydroxylated chalcone derivatives with variable chain length. *Natural Product Research, 26,* 892-902.

Ngaini, Z., Hui, D. H. A., Hussain, H., Zulkiplee, W. S. H. W., Tay, M. G., Sahari, N., & Nordin, N. A. (2013). Synthesis and antibacterial study of aspirin-chalcone derivatives. *Borneo Journal of Resource Science and Technology, 3*(1), 52-57.

Nordin, N. A., Lawai, V., Ngaini, Z., Abd Halim, A. N., Hwang, S. S., Linton, R. E., Lee, B. K., & Neilsen, P. M. (2018). In vitro cytotoxicity evaluation of thiourea derivatives bearing *Salix sp.* constituent against HK-1 cell lines. *Natural Product Research*, DOI: 10.1080/14786419.2018.1517120.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, *5*, e47, DOI: 10.1017/jns.2016.41.

Richard-Kortum, R. (2010). *Biomedical engineering for global health*. United States of America: Cambridge University Press.

Rubin, E., & Reisner, H.M. (2014). *Essentials of Rubin's pathology* (6th ed.). United States of America: Lippincott Williams & Wilkins.

Sie, C. Z. W., Ngaini, Z., Suhaili, N., & Madiahlagan, E. (2018). Synthesis of kojic ester derivatives as potential antibacterial agent. *Journal of Chemistry*, *2018*, DOI: 10.1155/2018/1245712.

Singhal, M., Paul, A., Singh, H.P., Dubey, S.K., & Songara, R.K. (2011). Synthesis and evaluation of antioxidant activity of semicarbazone derivatives. *International Journal of Pharmaceutical Sciences and Drug Research, 3*(2), 150-154.

Tsvetkova, B., Tencheva, J., & Peikov, P. (2006). Esterification of 7-theophyllineacetic acid with diethylene glycol monomethyl ether. *Acta Pharmaceutica, 56*, 251-257.

Vane, J. R., & Botting, R. M. (2003). The mechanism of action of aspirin. *Thrombosis Research, 110*, 255-258.

Weng, X. C., & Huang, Y. (2014). Relationship structure-antioxidant activity of hindered phenolic compounds. *Grasas Y Aceites, 65*(4), 1-8.

Williams, D. H., & Fleming, I. (1995). *Spectroscopic methods in organic chemistry* (5th ed.). England: McGraw-Hill Publishing Company.

Zhuang, C., Zhang, W., Sheng, C., Zhang, W., Xing, C., & Miao, Z. (2017). Chalcone: A privileged structure in medicinal chemistry. *Chemical Reviews*, *117*(12), 7762-7810.

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