Faculty of Resource Science and Technology

SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF ASPIRIN-CHALCONE DERIVATIVES

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Synthesis and Antibacterial Activities of Aspirin-Chalcone Derivatives

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DECLARATION

I, **Phang Sai Foong (38393), students from Resource Chemistry programme, Faculty of Resource Science and Technology** hereby declare that the work entitled **Synthesis and Antibacterial Activities of Aspirin-Chalcone Derivatives** is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

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(PHANG SAI FOONG)

Date :

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LIST OF ABBREVIATIONS

^{1}H NMR spectroscopy	Hydrogen Nuclear Magnetic Resonance
^{13}C NMR spectroscopy	Carbon-13 Nuclear Magnetic Resonance
$CDCl_3$	Chloroform
DMSO	Dimethyl sulfoxide
FTIR	Fourier Transform Infrared Spectroscopy
MIC	Minimum inhibitory concentrations
ppm	Part per million
TLC	Thin layer chromatography

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Synthesis and antibacterial activities of aspirin-chalcone derivatives

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Abstract

In recent years, chalcone and its derivatives have been studied extensively in terms of their biological activities. In this study, the synthesis and characterization of aspirin-chalcone derivatives has been carried out. A series of aspirin-chalcone derivatives were successfully synthesized from the reaction of intermediates **31a-c** with acetylsalicyloyl chloride to yield final product of **32a-c** with overall yield of 21.8 % to 88.4 %. All the derivatives were characterized by FT-IR, ^{1}HNMR and ^{13}CNMR. Disc diffusion method was then used to study the antibacterial activities of the new series. All of them showed the results of poor antibacterial properties. The structure that affected the strength of antibacterial properties was discussed.

Keyword: Aspirin, chalcone, aspirin derivatives, chalcone derivatives, antibacterial activities

Abstrak

*Dalam tahun-tahun kebelakangan ini, chalcone dan derivatifnya telah dikaji secara meluas dari segi aktiviti biologi mereka. Dalam kajian ini, sintesis dan pencirian derivatif aspirin - chalcone telah dijalankan. Satu siri derivatif aspirin - chalcone telah berjaya disintesis daripada reaksi **31a-c** dengan klorida acetylsalicyloyl untuk menghasilkan **32a-c** dengan hasil keseluruhan 21.8 % - 88.4 %. Semua derivatif telah dicirikan oleh FT- IR, ^{1}HNMR dan ^{13}CNMR . Kaedah cakera resapan telah digunakan untuk mengkaji aktiviti antibakteria siri baru. Semua produk menunjukkan keputusan ciri-ciri antibakteria yang lemah. Struktur yang menjejaskan kekuatan ciri-ciri antibakteria telah dibincangkan .*

Kata kunci : Aspirin , chalcone , derivatif aspirin , derivatif chalcone , aktiviti anti-bakteria

1.0 Introduction

1.1 Aspirin

Aspirin **1** is known as acetylsalicylic acid. It is a salicylate drug, which is used as an analgesic to relieve pains and minor aches. In addition, aspirin is antipyretic and always being applied to reduce fever and prescribed as an anti-inflammatory medication. For now, aspirin modifications have been conducted widely and many aspirin derivatives were proven to exhibit various biological properties such as antithrombic and antiplatelet (Lechi *et* al., 1996), anticancer (Lechi *et* al., 1996; Zheng *et* al., 2007) and antibacterial properties (Al-Bakri *et al.*, 2009). According to Karthikeyan *et al.* (2009), in the meta-analysis performed by the Antiplatelet Trialists' Collaboration, aspirin is reported to reduce the incidence of deep-vein thrombosis by 20% and that of pulmonary embolism by 69% in patients that have high risk for thromboembolic events.

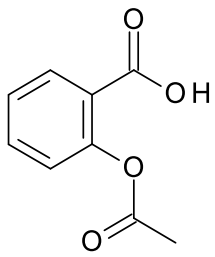

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Figure 1.1 : Structure of aspirin

1.2 Chalcone

Chalcone **2** is known as a group of compound consisting of two aromatic rings linked by an unsaturated α and β-ketone. There are various types of substituents on those two aromatic rings. Chalcone exists in most of the plants in nature. It is an intermediate precursor of

flavonoids and isoflavonoids. Chalcone has been applied widely in the field of biology and chemistry. Chalcone showed many biological properties including anticancer, antimalarial, antimicrobial, anti-inflammatory and antibacterial (Hsieh *et al*., 1998; Ram *et al*., 2000). In chemistry field, some studies showed that the photochemical and photophysical properties of chalcone enables it to be used as photoalignment and photocrosslinking unit in polymerization process, fluorescent dyes and light-emitting diodes (LEDs).

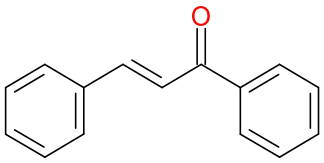

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Figure 1.2 : Structure of chalcone

1.3 Problem Statement

Currently, there are less studies conducted on synthesizing aspirin-chalcone derivatives. So, we would like to conduct this study by synthesizing aspirin-chalcone derivatives with various substituents. Through the structure modification, it is possible to create a product which contains even stronger antibacterial properties.

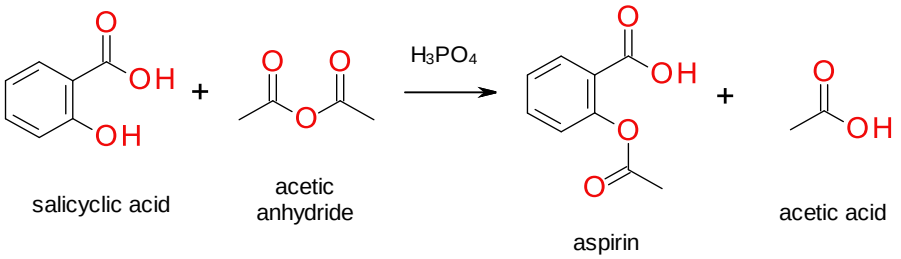
1.4 Objectives

1. To synthesize hydroxyl chalcone derivatives with long alkyl chain.
2. To incorporate hydroxyl chalcone derivatives onto aspirin.
3. To characterize the synthesized compounds using FTIR, ^{1}H and ^{13}C NMR.
4. To study the antibacterial activities of the synthesized compounds.

2.0 Literature Review

2.1 Aspirin

Aspirin is synthesized by reacting acetic anhydride and salicylic acid through esterification reaction. Phosphoric acid is applied as catalyst to speed up the reaction. At the end of the reaction, aspirin and acetic acid will be yield as main product and by product respectively.



Scheme 1 : Synthesis of aspirin

2.1.1 Recent study of aspirin derivatives

In the study carried out by Li *et al*. (2014), a novel aspirin derivatives, namely Ca-ASP **3** was prepared and characterized by reacting aspirin solution with hydroxylapatite (Hap) suspension. The anti-thrombotic and gastric mucosal protection properties study was carried out by using Winstar rats. Study shown that rats that consumed 5 mmol per kg body weight of Ca-ASP showed similar anti-thrombotic activity compared to those consumed same amount of aspirin, however, they suffered gastric mucosal damage in a much lower level compared to standard aspirin.

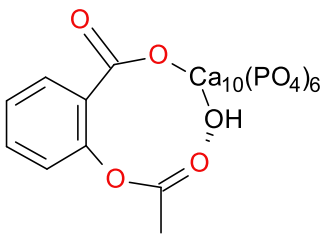


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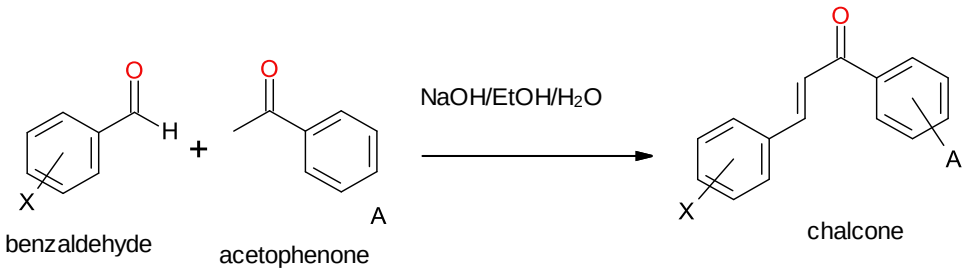
Figure 2.1 : Structure of Ca-ASP

In the study carried out by Balamani and Sekar (2011), simple new aspirin Enkephalin analogues were synthesized and evaluated for its analgesic activity studies. ACOH-inducing writhing test was then carried out on mice to evaluate their analgesic effects. The mean writhing values shown that Gly-Gly-Phe-Met-NH_2 sequence was important for the observed analgesic activity of compound Aspirin-Gly-Gly-Phe-Met-NH_2, while the Gly-Gly-Phe-Leu-NH_2 sequence was crucial for the observed analgesic activity of compound Aspirin-Gly-Gly-Phe-Leu-NH_2.

2.2 Chalcone

The general procedure to synthesis chalcone is by reacting equal molar of benzaldehyde and acetophenone in 95 % ethanol as a solvent. The mixture is stirred and cooled in an ice bath. To complete crystal the formation, it was filtered, wash in cold water and recrystallized with ethanol. The final product, chalcone is yielded.



Scheme 2 : Synthesis of chalcone

2.2.1 Antioxidant activity of chalcone derivatives

The antioxidant activity of 2'-*O*methylisoliquiritigenin **4**, isolated from the roots of *Dalbergia odorifera*, in lard was examined by Yu *et al*. (2007) by using oxidative stability instrument. As a result, greater antioxidant effects was obtained from this chalcone at 0.1 and 0.2 mM compared to butylated hydroxytoluene (BHT) and α-tocopherol. It was similar to α-tocopherol in inhibiting the decreasing of glutathione (GSH) level of rat lens induced by UV irradiation. The result could be useful to identify the mechanism of cataractogenesis.

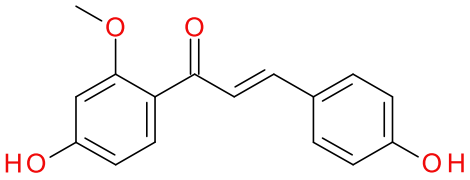


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Figure 2.2 : Structure of 2'-*O*methylisoliquiritigenin

From the study conducted by Mohamad *et al*. (2004), the 2',3',4',6'-tetrahydroxychalcone **5** isolated from dried ripe fruits of *Alpinia rafflesiana* exhibited more potent DPPH radical-scavenging activity (IC_{50} = 55 μM) compared to vitamin C (IC_{50} = 91 μM) and α-tocopherol (IC_{50} = 96 μM). From the result, chalcones were found out to be potent radical scavengers if it has catechol pattern of substitution in ring B and pyrogallol-type hydroxylated ring A.

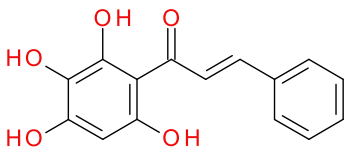

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Figure 2.3 : Structure of 2',3',4',6'-tetrahydroxychalcone

2.2.2 Anti-inflammatory activity of chalcone derivatives

Heme oxygenase 1 (HO-1) is known as crucial anti-inflammatory enzyme induced in response to oxidative stress and cytokines. A report by Sawle *et al*. (2008) stated that some methoxychalcones possessed anti-inflammatory activity that correlated with their potency as HO-1 inducers. By sequentially increasing the number of methoxy substituents in the 3,4,5- and 3',4',5'-positions of the aryl rings, a progressive increase in HO-1 activity was obtained. Meanwhile, Jin *et al*. (2007) also reported that by the induction of HO-1, which was initiated by depletion of GSH, 2',4',6'-tris(methoxymethoxy)chalcone **6** exhibited potent anti-inflammatory activity.

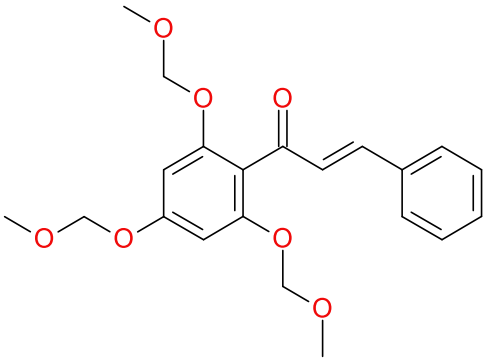

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Figure 2.4 : Structure of 2',4',6'-tris(methoxymethoxy)chalcone

The flavokawin A **7** isolated from *Piper methysticum* by Folmer *et al*. (2006), inhibited tumor necrosis factor (TNF)-α-induced IκBα degradation and translocation of p50 and p65 NF-κB subunits from the cytoplasm to the nucleus. Besides, important inflammation-related proteins such as p38-regulated/activated kinase, Iκβ kinase, Aurora B kinase, mitogen-activated protein kinase 3, dual-specificity tyrosine-phosphorylated and regulated kinase 1A, and also managed to be inhibited by flavokawin A.

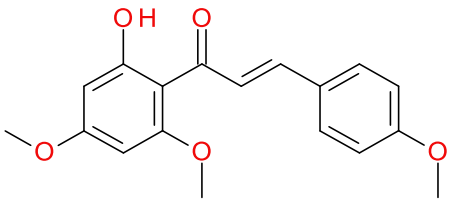

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Figure 2.5 : Structure of flavokawin A

2.2.3 Anticancer activity of chalcone derivatives

According to Amor *et al.* (2007), 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone **8**, isolated from *Syzygium samarangense* showed significant differential cytotoxicity against the adenocarcinoma SKBR-3 cell lines and human mammary MCF-7 with IC_{50} values of 12.8 x 10-4 nM and 15 x 10-4 nM respectively. Compared to former, the positive control doxorubicin had an IC_{50} value of 27.6x10-4 nM against the SKBR-3 cells and an IC_{50} value of 2.6 x 10-4 nM against the MCF-7 cell line.

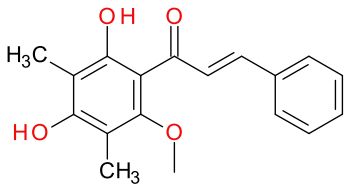

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Figure 2.6 : Structure of 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone

Kimura *et al*. (2004) isolated 4-Hydroxyderricin **9** from *Angelica keiskei*. Given orally at a dose of 50 mg/kg x 2/day, this compound successfully inhibited lung metastasis and prolonged the survival time in mice after the removal of subcutaneous tumors by surgical operation. This compound had additional properties by inhibiting the reduction of the numbers of lymphocytes, $CD4^+$, $CD8^+$ and natural killer (NK)-T cells in the spleen of tumor-removed mice (Kimura *et al*. 2004).

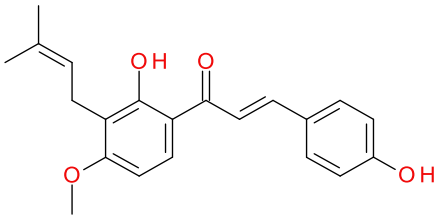

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Figure 2.7 : Structure of 4-Hydroxyderricin

2.2.4 Anti-infective activity of chalcone derivatives

2.2.4.1 Antibacterial activity

Compound **10** identified by Bowman *et al.* (2007) was concluded the most active against *S. aureus*, methicillin-resistant *S. aureus*, *S. epidermidis*, and *B. subtilis* with the MIC values 3.5, 3.0, 3.8, and 8.8 µM respectively. In comparison, the referent drug linezolid inhibited both *S. aureus* strains with recorded MIC values of 10.0 and 8.0 µM respectively.

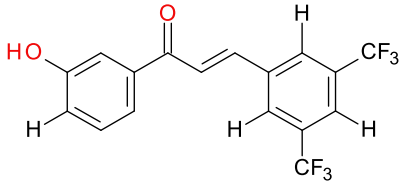

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Figure 2.8 : Structure of compound **10**

2.2.4.2 Antifungal activity

Mbaveng *et al*. (2008) screened isobavachalcone **11** for its antifungal activity, and found out that it inhibited growth of *Microsporum audorium* (MIC = 0.3 µg/ml), *Candida albicans* (MIC = 0.3 µg/ml), *C. glabrata* (MIC = 0.3 µg/ml) and *Trichophyton rubrum* (MIC = 1.2 µg/ml).

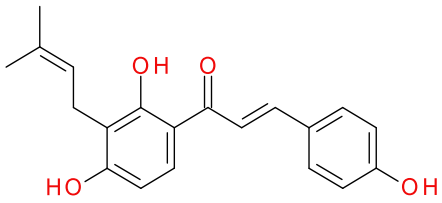

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Figure 2.9 : Structure of isobavachalcone

2.2.4.3 Antiviral activity

An attempt was made by Deng *et al.* to develop small molecules to inhibit HIV-1 integrase (IN), a crucial enzyme for viral replication. Compound **12** was synthesized successfully and in the presence of both Mn^{2+} and Mg^{2+} as cofactors, it showed potent inhibitory activity with an IC_{50} value of 2 μM against purified IN.

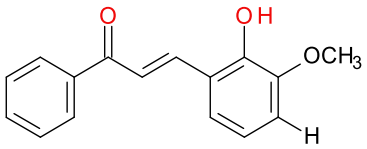

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Figure 2.10 : Structure of compound **12**

2.2.4.4 Antiparasitic activity

Licochalcone A **13**, the well-known antiparasitic compound is a potent membrane-active agent that undergoes transformation of normal erythrocytes into echinocytes in parallel with the inhibition of growth of *P. falciparum* cultures. Ziegler *et al*. (2004) managed to observed the erythrocyte membrane-modifying effect transiently in mice after intravenous administration.

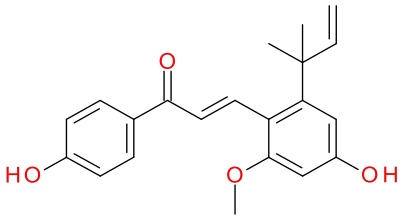

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Figure 2.11 : Structure of licochalcone

2.2.5 Recent studies of chalcone derivatives

In the study conducted by Singh *et al*. (2010), a new pharmacophore named 'Chalconesemicarbazone' was designed by pharmacophore hybridization of drug design. Singh *et al*. (2010) a series of novel 'chalconylsemicarbazide' derivatives by reacting substituted phenyl semicarbazide with various chalcone derivatives, which contained hydrogen acceptor site, hydrogen donor site and lipophilic site which may aid in receptors binding for pharmacological activities. Carrageenan-induced rat paw edema test was used to conduct *in vivo* determination of the anti-inflammatory activity. Meanwhile, to evaluate the analgesic activity, acetic acid-induced writhing test was used. Finally, the percentage of protection against the acetic acid-induced writhing was determined and calculated. The screening result shown that 1-(1,5-diphenylpenta-2,4-dienylidene)-4-(2-nitrophenyl) semicarbazide was most active.