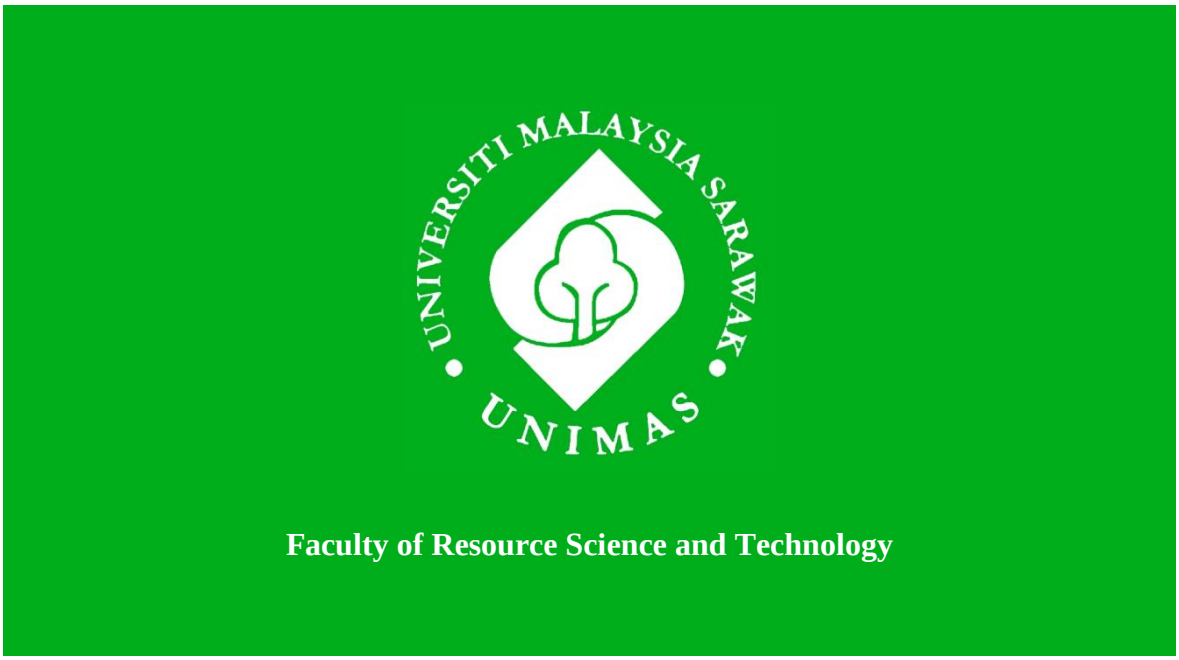


SYNTHESIS OF ASPIRIN-CHALCONE DERIVATIVES AND THIER ANTIBACTERIAL ACTIVITY

LIM JI YUAN 36720

Bachelor of Science with Honours

Resource Chemistry

2015

Synthesis of Aspirin-Chalcone Derivatives and Their Antibacterial Activity

Lim Ji Yuan

A dissertation is submitted in partital fulfilment of the requirement for the Degree of Bachelor Science with Honours

(Resource Chemistry)

Faculty Resource Science and Technology

Universiti Malaysia Sarawak

2015

ACKNOWLEDGEMENT

First of all, I would like to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Zainab Ngaini, for giving me the opportunity to conduct this research title for my final year project. Her patience guidance and generous help give me a lot of support in completing this thesis.

I am grateful to have all the postgraduate students in the organic laboratory. I would like to thank them for sharing their experience and giving suggestions to help me in overcoming obstacles during my research.

I'm going to take this opportunity to thank to all the technical staff and laboratory assistant for their proficient assistance and providing laboratory facilities. I would also like to thank to my fellow friends for supporting me during this research.

Last but not least, I also thank to my family members who always give their fully support to me, encourage me in my study. In addition, my sense of gratitude to one and all, who direct or indirectly lent their hand in this venture.

DECLARATION

In this dissertation, there is no part of work has been submitted in report of an application for another degree of qualification of this or any other university or institution of higher learning. I declare that this project is the work of my own excluded of the references document that have been acknowledged.

……...

(LIM JI YUAN)

Resource Chemistry

Department of Chemistry

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

TABLE OF CONTENT

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
MHA	Muller Hinton agar
MIC	Minimum inhibitory concentrations
MRSA	*Staphylococcus aureus* ATCC 43300
MSSA	*Staphylococcus aureus* ATCC 29213
ppm	Part per million
QSAR	Quantitative structure-activity relationships
rotavap	Rotatory evaporator
TLC	Thin layer chromatography

LIST OF TABLES, FIGURES AND SCHEMES

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

Synthesis of Aspirin-Chalcone Derivatives and its Antibacterial Activity

Lim Ji Yuan

Department of Chemistry

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

ABSTRACT

Aspirin and chalcone have long been used for its medicinal purpose due to its biological activity. This research focused on the synthesis of aspirin-chalcone derivatives resulting from the reactions of chalcone intermediate with acetylsalicyloyl chloride. Aspirin-chalcone derivatives have been synthesized. The successfully synthesized compounds were characterized using FTIR, ^{1}H NMR and ^{13}C NMR. Antibacterial assay was carried out using disc diffusion method against the growth of *Escherichia coli* ATCC 29522. However, the result indicated that the newly synthesized aspirin-chalcone derivatives showed no antibacterial activity against *E.coli*.

Keywords: Aspirin-chalcone derivatives, Antibacterial activity, *E.coli*

ABSTRAK

Aspirin dan chalcone telah lama digunakan untuk tujuan perubatan disebabkan oleh aktiviti biologi. Kajian ini memberi tumpuan kepada sintesis derivatif aspirin-chalcone hasil daripada reaksi chalcone perantaraan dengan klorida acetylsalicyloyl. Derivatif Aspirin-chalcone telah disintesis. Sebatian berjaya disintesis telah dicirikan menggunakan FTIR, ^{1}H NMR dan ^{13}C NMR. Kajian antibakteria telah dijalankan dengan menggunakan kaedah cakera resapan terhadap pertumbuhan Escherichia coli ATCC 29522. Walau bagaimanapun, hasilnya menunjukkan bahawa derivatif aspirin - chalcone baru disintesis menunjukkan tiada aktiviti antibakteria terhadap E.coli.

Kata kunci: Derivatif aspirin-chalcone, aktiviti anti-bakteria, E.coli

CHAPTER 1

INTRODUCTION

1.1 Aspirin

The discovery of aspirin in the early 1900s had make a major impact in the medical industries. Aspirin can be used in the treatment under these conditions including fever, rheumatic fever, pain and inflammatory diseases. Aspirin is known to be one of the most widely used medications in the world, with an estimated 40 000 tonnes of it being consumed each year (Warner & Mitchell, 2002). In the United States alone, it has been estimated that approximately 20,000 million aspirin tablets are consumed per annum and this represents about 100 tablets/year for every man, woman and child (Taylor, 1981). The fact that aspirin has remained so popular for so long shows its acceptability to the general public and a good indication of its therapeutic usefulness for treating minor aches and pain.

Aspirin is a salicylate drug, which used as an analgesic, antipyretic and anti-inflammatory medications. 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). Aspirin still recognised as the standard analgesic, anti-inflammatory and antipyretic agent where newfound drugs are developed based on it. However, according to Clissold (1986), its leading position as the analgesic of the choice has been gravely challenged with the emergence of other ‘new’ non-steroidal non-narcotic analgesic drugs such as diflunisal, flurbiprofen and ibuprofen are significantly greater analgesics and have a longer duration of action.

Clissold (1986) mentioned that aspirin is generally considered safe by most people, it is certainly not harmful, side effects, especially gastrointestinal disturbances. Additionally, aspirin is a common cause of analgesic poisoning in both adults and children. Accidental overdosing, is probably a reflection of the drug's popularity and wide availability, and the population's acceptability of its efficacy and presumed safety.

1.2 Chalcone

Chalcone is a compound that contain two aromatic rings linked by an unsaturated α, β – ketone, with various substituents on the two rings. Chalcone is a natural pigment which could be easily found in most of the plants and it is a vital intermediate precursor of flavonoids and isoflavonoids (Ngaini *et al.*, 2012; Ha & Low, 2013). Their numerous biological activities have lead many researches to study on chalcone in the recent decades. Compounds with chalcone as the backbone have been stated to show different biological activities such as anticancer (Ngaini *et al.*, 2012), antitumor, anti-tubercular, antileishmanial (Ha & Low, 2013) antioxidant, antimicrobial (Tiwari *et al.*, 2010; Ngaini *et al.*, 2012), anti-inflammatory and antimalarial (Ha & Low, 2013; Tiwari *et al.*, 2010). Chalcone is also reported to have photophysical and photochemical properties, for example it was being used as fluorescent dyes, photocrosslinking and photocrossalignment unit in polymerization process, light-emitting diodes and many more (Ha & Low, 2013).

The chemistry of chalcone has been recognized as a significant field of study. The extraordinary growth of publications in this area reflects the interest in this field throughout the world. One of the interesting feature of chalcones is that they serve as a raw material for the synthesis of various heterocyclic compounds such as pyrazolines, pyrimidines, flavonols, flavones, flavanones, aurones and benzoylcoumarones as well as

certain compounds like hydantions and deoxybenzoins which are of some therapeutic importance.

Aspirin-chalcone derivatives have been carried out to study the antibacterial property against certain bacteria (Ngaini *et al.*, 2013). This finding has stimulated our interest in exploring more applications on aspirin-chalcone derivatives.

In this research, the starting material, 4-hydroxyacetophenone was going to react with a series of 1-bromoalkane. Then, the 4-alkoylacetophenone was further react with 4-hydroxybenzaldehyde *via* Claisen-Schmidt condensation to form chalcone derivatives. Acetylsalicyloyl chloride and chalcone derivatives were add together to form the aspirin-chalcone derivatives.

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. It is possible to create a product which enhance its antibacterial property.

1.4 Objectives

The main objectives of this research are:

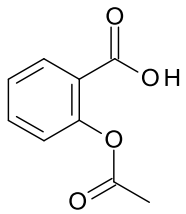
1. To incorporate different length of alkyl group, C_nH_{2n+1} where n = 10, 12, 14 onto 4-hydroxyacetophenone
2. To synthesize chalcone and aspirin-chalcone derivatives
3. To characterize aspirin-chalcone derivatives using FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy
4. To study the antibacterial activity of aspirin-chalcone derivatives

CHAPTER 2

LITERATURE REVIEW

2.1 Aspirin

Aspirin can be considered as one of the most widely used medical care in the world. Aspirin or acetylsalicylic acid, is a salicylate drug. It was used as analgesic to relieve pains and aches, as an antipyretic that lowers body temperature to alleviate fever and as an anti-inflammatory medications. It is a white, crystalline, weakly acidic substance. It has a boiling point of 140°C and a melting point of 136°C. Its acid dissociation constant, pKa is 3.5 at 25°C. Lower doses of aspirin have also shown to help prevent blood clot formation, heart attacks and strokes. Alteration of aspirin have been carried out and many aspirin derivatives were reported to show various biological activities for example antimicrobial, anticancer, antiplatelet and antithrombic.



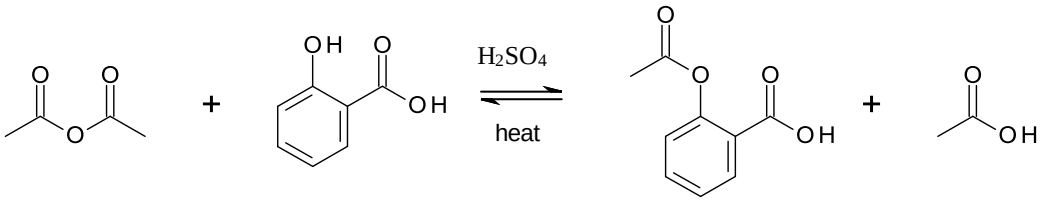
4

Figure 2.1 Structure of aspirin

2.1.1 Preparation of Aspirin

The synthesis of aspirin undergoes esterification reaction. Acetic anhydride is reacted with salicylic acid, causing a chemical reaction that changes salicylic acid's hydroxyl group into

an ester group. This reaction forms aspirin and acetic acid, which is considered as a byproduct. Small amounts of sulphuric acid, H_2SO_4, are almost always used as a catalyst.

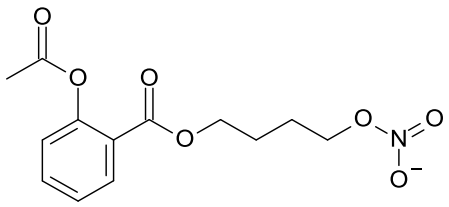


Scheme 1. Synthesis of aspirin

2.1.2 Aspirin Derivatives

2.1.2.1 Anti-thrombotic Property

The anti-thrombotic actions of aspirin are related to its ability to control thromboxane synthesis, by inhibiting the enzyme cyclo-oxygenase in platelets. However, the suppression of cyclo-oxygenase is associated with significant toxicity in the gastrointestinal tract and less frequently in kidney. This toxicity limits the usage of aspirin of long-term use as an anti-thrombotic agent. In Wallace *et al*. (1995) studies, they have compared aspirin to nitric oxide-releasing aspirin derivatives (NCX 4215) **5** in terms of their ability to inhibit platelet aggregation in vitro and ex vivo, their effects on prostaglandin and thromboxane synthesis and their ulcerogenic properties in stomach.

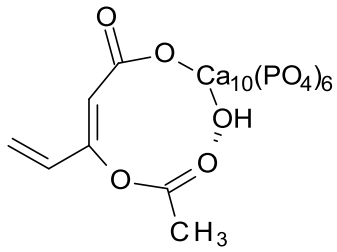


5

Figure 2.2 Structure of NCX 4215 (acetylsalicylic acid 4-(nitroxy)butylester)

This study was carried out on human platelet *in vitro*, *in vivo* and *ex vivo* studies on rat. Wallace *et al.* (1995) stated that compound **5** was shown to be about seven times more influential than aspirin as an inhibitor of thrombin-induced human platelet aggregation in vitro, but the gastric prostaglandin synthesis or platelet thromboxane synthesis was not inhibited. When compound **5** was incubated in the presence of platelets and increased platelet levels of cGMP within 10 minutes of exposure, it released nitric oxide whereas aspirin doesn't. 10 μM haemoglobin significantly reduce the anti-aggregatory effects of compound **5** *in vitro*. Aspirin and compound **5** shown similar inhibitory effects 3 hours after medication in the *ex vivo* studies of thrombin-induced rat platelet aggregation or collagen- or ADP-. Aspirin (10-120 mg/kg) had caused extensive haemorrhagic erosion formation in the stomach of the rat within 3 hours of oral medication, while compound **5** did not show significant damage at doses up to 300 mg/kg, neither when given daily for two weeks at 166 mg/kg. Compound **5** did not change systemic arterial blood pressure when administered endovenously to the rat. These studies shown that compound **5** has similar or improved anti-thrombotic activity to that of aspirin, but does not modify systemic blood pressure or cause gastric damage. The anti-thrombotic actions of compound **5** are due to generation of nitric oxide.

In another study, Li *et al.* (2014) shows the aspirin derivative possess anti-thrombotic and also gastric mucosal protection properties. They have synthesized a derivative form of aspirin, prepared by modifications of aspirin with nano-hydroxyapatite (a kind of inorganic particle containing Ca^{2+}). Hydroxyapatite (Hap) is a component of the bones and has good biocompatibility and low cytotoxicity, and it's has been used as delivery vehicles for different drugs and genes (Li *et al.*, 2014). This compounds was named Ca-ASP **6**.



6

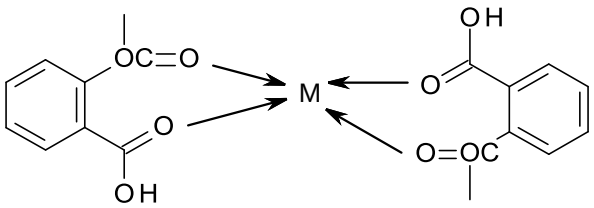
Figure 2.3 Structure of Ca-ASP

In this experiment, thirty rats were randomly divided into five groups (six animals per group). The control group was administered 0.5% Carboxyl Methylated Cellulose (CMC) aqueous solution, while aspirin group was administered aspirin (dissolved in 0.5% CMC aqueous solution) at a dose of 0.5 mmol/kg of body weight. The remaining three groups were each given a different dosage of Hap-modified aspirin (0.1, 0.25 or 0.5 mmol/kg, dissolved in 0.5% CMC aqueous before gavage). According to Li *et al.* (2014), rats were given a high dose of compound **6** (5 mmol/kg body weight) showed similar anti-thrombotic activity as those given the same dose of aspirin, but had much lower gastric mucosal damage than aspirin. These rats also showed reduced expression of COX-2, but their COX-1 expression was similar to that of control rats, but significantly higher than that of aspirin-administered rats. The level of prostaglandin 2 (PGE2) was up-regulated in compound **6**-administered rats compared to ASP-administered rats. Furthermore, the modifications of aspirin by Hap converted the free carboxyl (COOH) group of aspirin to a salt form, making it less reactive, and the drug therefore caused no local lesion to the gastric mucosa.

2.1.2.2 Antibacterial property

In the research of Obaleye and Lawal (2007), the synthesis of novel transition metal complexes of aspirin and paracetamol were subjected to antibacterial activity testing. The

test compounds was assayed against three bacteria: *Bacillus Subtilis, Serratia Species* and *Escherichia coli*. The antibacterial activity was determined on the seeded nutrient agar on which 0.9 cm diameter wells punched. The sterile filtered solutions of the ligands with different concentrations (0.1% and 1.0% w/v) and the complexes were made using methanol as solvent. 0.1 ml of each concentration was applied into the wells and incubated at 37ºC for one to three days. The antibacterial activity was estimated based on the size of inhibition zone formed around the well of the seeded agar plates and the inhibition growth in percentage was calculated on the basis of the average diameter of bacterial colony on the growth medium to their respective controls as in the equation, % inhibition = $\frac{A-B}{A}$ x 100.



7

Figure 2.4 Aspirin-metal complexes, $M(Asp)_2X_n$

Aspirin-metal complexes that were synthesized are $Co(Asp)_2Cl_2$, $Ni(Asp)_2Cl_2$ and $Fe(Asp)_2Cl_3$. Obaleye and Lawal (2006) mentioned that the aspirin complexes doesn't show any effect on *Serratia* and *E.coli* species at both concentrations. The aspirin complexes that showed the greatest inhibitory effect against *Bacillus subtilis* was $Co(Asp)_2Cl_2$, whereas $Ni(Asp)_2Cl_2$ have the lowest.

2.1.2.3 Antiplatelet property

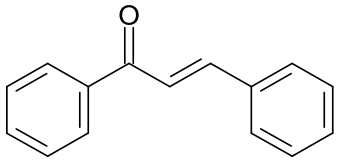
Furthermore, the studies of Guthikonda *et al.* (2006) mentioned that the mechanisms for the variability in antiplatelet effects of aspirin was still not clear. The reticulated platelets may change the antiplatelet effects of aspirin through uninhibited cyclooxygenase COX-1

and COX-2. Therefore, this research was carried out to evaluate the role of reticulated platelets effects of aspirin. Platelet studies included light transmission aggregometry, P-selectin and integrin $\alpha_{IIb}\beta_3$ expression and serum thromboxane B_2 (TxB_2) levels, were performed against 60 healthy volunteers before and 24 hours after injection of a single 325-mg dose of aspirin. The results showed that the reticulated platelets reduced the antiplatelet effects of aspirin and it also increased the aspirin resistance. This may be due to the increased reactivity and uninhibited COX-1 and COX-2 activity.

2.2 Chalcone

The intensive scientific studies on chalcone have been carried out throughout the world. The synthesis and biodynamic activities of chalcone become the focus of many researchers' interest. Patel (2011) stated that the name "chalcone" was given by Kostanecki and Tambor. The alternative name given are benzalaacetophenone, phenyl stryl ketone, *α*-phenyl-*β*-benzoylethylene, *β*-phenylacrylophenone, and *γ*-oxo-*α*,*γ*-diphenyl-*α*-propylene. Chalcone is an aromatic ketone that forms the central core for the variety of important biological compounds (Kromann & Feldbaek, 2004). At the terminal position of the system – C = C – C = O – whereby two aromatic substituent are introduced to form this compound. They are characterized by their position of an Ar(A)-CO-CH=CH-Ar(B) in which two aromatic rings A and B are linked by an aliphatic three carbon chain. Chalcone is classified under flavonoid family. It contains an open-chain flavonoids where two aromatic rings are joined by three-carbon α, β unsaturated carbonyl system. It is also α, β unsaturated ketones containing the reactive keto-ethylenic group, - CO – CH = CH - . Chalcone is a coloured compounds due to the existence of the chromophore – CO – CH = CH - , which rely on the presence of other auxochromes. They shows few biological activities such as antimicrobial, antioxidant, antibacterial and so forth. Chalcone and their derivatives find applications as

artificial sweeteners, scintillator, polymerisation catalyst and fluorescent whitening agent, and organic brightening agent, stabilizer against heat, visible light, ultra-violet light and aging.

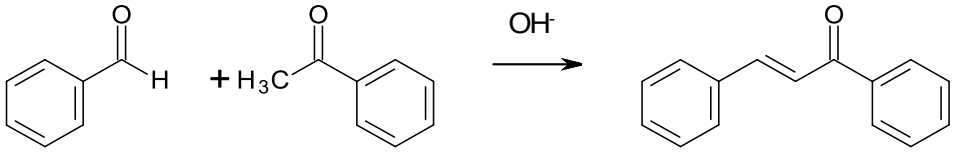


8

Figure 2.5 Structure of chalcone

2.2.2 Preparation of Chalcone

There are different methods accessible for the preparation of chalcones. Claisen-Schimdt condensation is the most common method with arylmethylketone and aryl aldehyde react together in the presence of alcoholic alkali.



Scheme 2. Synthesis of chalcone

2.2.3 Importance of Chalcone

2.2.3.1 Antibacterial Property of Chalcone

In Cushnie and Lamb (2005) studies, the antimicrobial activity of these compounds mainly attributed to the presence of phenolic hydroxyl groups, phenyl rings, and α,β-unsaturated ketones or benzopyrone rings, which have high affinity to proteins and thus may inhibit

microbial enzymes. According to Abdula (2013), the research is on the synthesis of chalcone and screening their activities against some Gram-negative and Gram-positive bacterial species. *Klebsiella SPP, Escherichia coli* (Gram-negative) and *Enterococcus faecalis, Staphylococcus aureus* (Gram-positive) were tested for antibacterial activity on chalcone derivatives using well diffusion method. The results revealed that the chalcone derivatives exhibited moderate to potent bacterial growth inhibition against some Gram-negative and Gram-positive strains. In Sivakumar *et al.* (2009) studies shows that 48 chalcone analogues were synthesized and their in vitro antibacterial activity against *Bacillus subtilis* NCIM 2718, *Enterobacter aerogenes* NCIM 5139, *Escherichia coli* NCIM 2931, *Phaseolus vulgaris* NCIM 2813, *Salmonella typhi* 2501 and *Staphylococcus aureus* NCIM 5021 were assessed by microdilution broth assay. Quantitative structure-activity relationships (QSAR) were developed for all the cases. The activity against these Gram-negative and Gram-positive bacteria were determined by hydrophilic nature of the molecule, electron-donating or withdrawing, polarizability and size. *Staphylococcus aureus* was the most and *Salmonella typhi* was the least hydrophobic of these organisms. These chalcones act better against more hydrophobic organisms.

In Thai *et al.* (2012) study, a series of heterocyclic chalcones analogues with one of the benzene ring replaced by the electron rich nitrogen, oxygen and thiophene heterocycle have been synthesized and evaluated for its antibacterial activity. The test were performed by dilution method using a vancomycin and methicillin resistant *Staphylococcus aureus* separated from a human sample, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA) and *Staphylococcus aureus* ATCC 29213 (MSSA or SA). Out of 21 synthesized chalcones, only 5 compounds exhibited strong activity against the tested *Staphylococcus aureus*.