Faculty of Resource Science and Technology

SYNTHESIS OF BENZALDEHYDE DERIVATIVES AS ANTIOXIDANT AGENT ASSISTED BY PHARMACOPHORE MODELLING (*Acne vulgaris*)

Nurul Amani Binti Mohd Zaini
57488

Bachelor of Science with Honours
(Resource Chemistry)
2019

UNIVERSITI MALAYSIA SARAWAK

Grade: A

Please tick ( )

Final Year Project Report ✓

Masters

PhD

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This declaration is made on the 28th day of May year 2019

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28th May 2019
Date submitted

NURUL AMANI BINTI MOHD ZAINI (57488)
Name of the student (Matric No.)

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SYNTHESIS OF BENZALDEHYDE DERIVATIVES AS ANTIOXIDANT AGENT ASSISTED BY PHARMACOPHORE MODELLING (*Acne vulgaris*)

Nurul Amani Binti Mohd Zaini (57488)

This Final Year Project is submitted in partial fulfillment of the degree of

Bachelor of Sciences with Honours

(Resource Chemistry)

Supervisor : Dr. Mohd. Razip bin Asaruddin

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

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ACKNOWLEDGEMENT

I would like to express greatest gratitude to my supervisor, Dr. Mohd Razip Asaruddin for his guidance and advice to complete this final year project. Special thanks to my parents and family for their moral support. I also would like to thank my friends and my labmates for their time, energy and for being really helpful and supportive. I also would like to dedicate my appreciation to the postgraduates students for guiding me to complete this project. Last but not least, thanks to other people that involve directly or indirectly in this project.

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List of Abbreviations

Reactive Oxygen Species	**ROS**
Superoxide Dismutase	**SOD**
Malondialdehyde	**MDA**
Computer-Aided Drug Design	**CADD**
Fourier Transform Infrared Spectroscopy	**FTIR**
Thin Layer Chromatography Technique	**TLC**
Nuclear Magnetic Resonance	**NMR**
Ligand-Based Drug Design	**LBDD**
Hydrogen Bond Acceptors	**HBA**
Hydrogen Bond Donors	**HBD**
Hydrophobic Interaction	**Hy**
Negative Ionizable	**NI**
Positive Ionizable	**PI**
Ethanol	**EtOH**
Lethal Concentration for 50%	**LC_{50}**
Inhibition Concentration for 50%	**IC_{50}**

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ABSTRACT

Acne Vulgaris is a common chronic inflammatory skin disease which affecting adolescents and often continuing into adulthood. This pilosebaceous units of the skin affected and causes inflammatory or non-inflammatory lesions. The development of cutaneous inflammatory diseases is caused by inadequate antioxidant protection and or excess reactive oxygen species (ROS) production that creates a condition known as oxidative stress. This project was carried out to synthesize anti-oxidant agent for acne vulgaris through Schiff base reaction and pharmacophore modelling by using benzaldehyde derivatives. Compound that has been synthesized was characterized by Fourier Transform Infrared Spectroscopy (FTIR), Thin Layer Chromatography (TLC) technique and Nuclear Magnetic Resonance ^{1}H NMR. This project was facilitated by using pharmacophore modelling to search anti-acne drugs. Pharmacophore model was developed by using Liganscout 4.3 software. The pharmacophore model is developed from three training sets that was selected from established ant-acne drugs. The best model was validated with 10 test sets from the synthesized compound. The validation shows that compound 10 and compound 9 has the highest pharmacophore fit-value. The cytotoxicity assay using brine shrimp (*Artemia salina*) and antioxidant assay using diphenylpicrylhydrazyl (DPPH) radical was conducted. From the results, compound 9 is the most effective anti-oxidant agent than the other compounds.

Keywords : Acne vulgaris, pharmacophore, benzaldehyde, Schiff base, *Artemia salina*, antioxidant

ABSTRAK

Jerawat Vulgaris adalah penyakit kulit keradangan kronik yang biasa yang mempengaruhi remaja dan sering berlarutan sehingga ke masa dewasa. Unit-unit pilosebase kulit yang terjejas akan menyebabkan luka keradangan atau tidak radang. Perkembangan penyakit radang kulit adalah disebabkan oleh perlindungan antioksidan yang tidak mencukupi dan pengeluaran spesis oksigen reaktif (ROS) yang berlebihan menghasilkan keadaan yang dikenali sebagai tekanan oksidatif. Projek ini dijalankan untuk mensintesis agen anti-oksidan untuk jerawat vulgaris melalui reaksi Schiff Base dan pemodelan farmakofor dengan menggunakan derivatif benzaldehid. Kompaun yang telah disintesis dicirikan oleh teknik Fourier Transform Infrared Spectroscopy (FTIR), teknik Thin Layer Chromatography (TLC) dan Resonansi Magnet Nuklear ^{1}H NMR. Projek ini difasilitasi dengan menggunakan pemodelan farmakofor untuk mencari ubat anti-jerawat. Model farmakofor telah dibangunkan dengan menggunakan perisian Ligandscout 4.3. Model farmakofor dikembangkan dari tiga set latihan yang dipilih dari ubat anti jerawat yang telah ditetapkan. Model terbaik disahkan dengan 10 set ujian dari sebatian yang disintesis. Pengesahan menunjukkan bahawa kompaun 10 dan kompaun 9 mempunyai nilai fitakfosit tertinggi. Pengujian sitotoksisiti menggunakan udang air garam (Artemia salina) dan ujian antioksidan menggunakan radikal diphenylpicrylhydrazyl (DPPH) telah dijalankan. Dari hasilnya, sebatian 9 adalah agen antioksidan yang paling berkesan daripada sebatian lain.

***Kata kunci**: Acne vulgaris, farmakofor, benzaldehyde, asas Schiff, Artemia salina, antioksidan*

1.0 Introduction

Acne vulgaris is a common chronic inflammatory skin disease. 80% of young adults and adolescents are found to be suffering from this disease. This disease affects the pilosebaceous units of the skin and causes inflammatory or non-inflammatory lesions (Dessinioti *et al*.,2010). Strauss e*t al*., (2007), defined acne as a chronic inflammatory dermatosis which consists of open comedones (blackheads), closed comedones (whiteheads) and inflammatory lesions such as nodules, pustules and papules. In recent years, acne has been observed in younger patients due to the earlier onset of puberty (Lavers.,2014). Acne has many negative effects on young adolescents. It causes discomfort, emotional stress, disfigurement and even permanent scarring to the skin (Feldman *et al*.,2004). Several factors may trigger acne production or increase its severity which include genetics, the male sex, youth, stress and smoking as well as comedogenic medications such as androgens, halogens, corticosteroids and pore clogging cosmetics.

Figure 1 : Image of acne on human skin

In recent years, long term use of antibiotics against acne is outdated because of exacerbated antibiotic resistance (Degroot *et al*., 1998). There has been an increasing focus on the extent to which oxidative stress is involved in the pathophysiology of acne. Emerging studies have shown that patients with acne are under increased cutaneous and systemic oxidative stress (Sarici *et al*.,2007).

This project was carried out to design and synthesis anti-oxidant agent for acne vulgaris through schiff base reaction and pharmacophore modelling. The objectives of this project are to design alternative drugs for acne by using anti-oxidant agent from Schiff base containing benzaldehyde using pharmacophore modelling and to synthesize the designated compounds via Schiff base reaction based on modelling simulation outcomes. The objectives of this project also to evaluate the cytotoxicity and the effectiveness of the synthesized compounds

2.0 Literature Review

2.1 Acne Vulgaris

Acne vulgaris is a common skin disease affecting more than 85% of adolescents and often continuing into adulthood (Collier *et al*.,2008). Acne comprises lesions of various morphology, from comedones, papules, and pustules to nodules and cysts (Amin *et al.*,2007). Acne affects the pilosebaceous units of the skin which presents with a variety of lesions at various inflammatory stages, including acne scars and hyperpigmentation (Webster *et al*.,2002). Acne lesions are most commonly present on the face, chest, upper back and upper arms which are known to have a high density of sebaceous glands (Olutunmbi e*t al*., 2008). The four main pathological factors involved in the development of acne are the increased sebum production, irregular follicular desquamation, Propionibacterium acnes proliferation and inflammation of area (Gollnick *et al*.,2003).

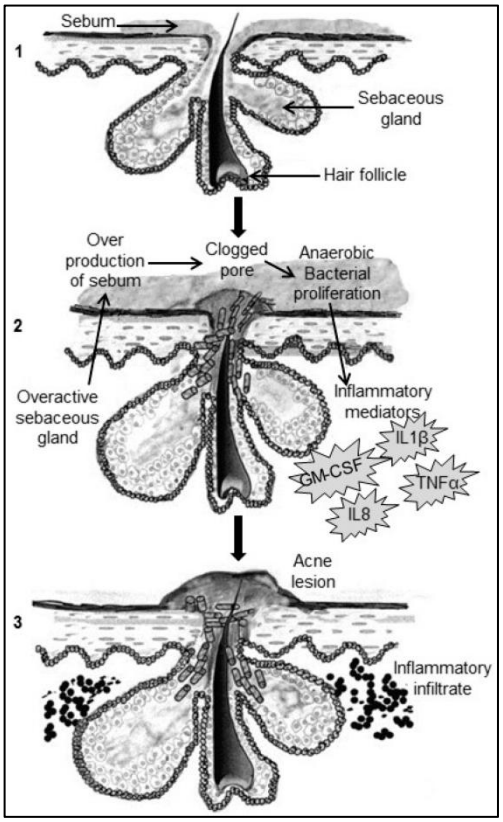


Figure 2 : Pathogenic factors contributing to the development of acne from (Muizzudin *et al.*,2008)

Recent studies on the etiopathogenesis of acne vulgaris have focused on the role of oxygen free radicals and antioxidant enzymes (Bickers *et al.*,2006). Inadequate antioxidant protection and⁄or excess reactive oxygen species (ROS) production creates a condition known as oxidative stress, which contributes toward the development of cutaneous inflammatory diseases (Briganti *et al*.,2003). It has been reported that oxygen free radicals, which are generated by the neutrophils on the follicular wall to kill microorganisms, may cause cell damage at the site of inflammation (Akamatsu *et al*.,2003). Sebum composition is altered in acne and ROS produced by neutrophils are involved in the irritation and destruction of the follicular wall that is responsible for the inflammatory progression of acne (Thiele *et al*.,1999). Superoxide dismutase (SOD) is a group of metalloenzymes that scavenges superoxide radicals and reduces their toxicity. It is an antioxidant that dismutates the O^{2-} anion to form O_2 and H_2O_2 (Koca *et al*.,2004). Malondialdehyde (MDA) is the end product of lipid peroxidation. If antioxidant enzymes become incapable of dealing with the oxidative damage, oxygen free radicals initiate lipid peroxidation in cell and organelle membranes (Arican *et al*.,2005). MDA is a good marker of free radical-mediated damage and oxidative stress (Del Rio *et al*.,2005).

2.2 Benzaldehyde

Benzaldehyde is a chemical compound that made up from benzene and carbonyl group. It is colorless, aromatic liquid that has a pleasant almond-like odor. It quickly evaporates from liquid to gas when it is expose to the air. Benzaldehyde is mainly used as a food and flavoring additive and can be found in many foods such as frozen dairy, baked goods, fruit juice, soft candy, gelatin pudding, non-alcoholic beverages, chewing gun, alcoholic beverages and hard candy (Feneroli,2005). Benzaldehyde has been used as carcinostatic drug that slow down or inhibits the growth of cancerous tumors in humans and lab animals (Liu *et al*.,2008). Patlewicz et al., (2001) concluded that benzaldehyde is not a skin-sensitizer based on the available

laboratory data and a detailed consideration of chemistry involved in dermal reactions. Benzaldehyde also has been used in pharmaceuticals such as drugs, fragrances such as perfumes and deodarants, dyes, personal care items such as moisturizing creams, as additive and as artificial flavoring. Benzaldehyde also used as solvent including oils, cellulose fibres and resins (Bureau of Environmental Health, 2013).

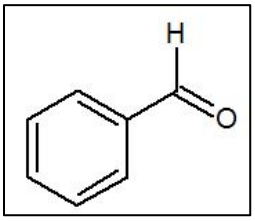


Figure 3 : Structure of benzaldehyde

2.3 Schiff Base Reaction

In this project, synthesis of benzaldehyde will be done by using Schiff Base mechanism. Schiff base are the compound that contains azomethine group (-HC=N-). The compounds are produced by the condesation reaction of either ketones or aldehydes, or both with primary amines (Worku *et al*.,2002). Schiff bases have been used as chelating ligands in anti-oxidative activity (Yildiz *et al*.,2004). Benzaldehyde derivatives contain imine group have a biological properties of inhibitory effect. It is widely used as antimicrobial agent because of its inhibitory effect as bacteriocide, fungicide, and algaecide (Subramanyam *et al*.,2009).

2.4 Pharmacophore Modelling

Pharmacophore modeling approach is a computer-aided drug design (CADD) method which possessed potential as the most promising candidates to focus on the experimental efforts in modern medicinal chemistry. Pharmacophoric information will be provided for future development of more potent molecules in the series of phenyl pyrazines and determine the structural and molecular properties (Kaur *et al.,* 2012). Computer-Aided Drug Design (CADD) is a computational knowledge-based methods to aid the drug discovery process.

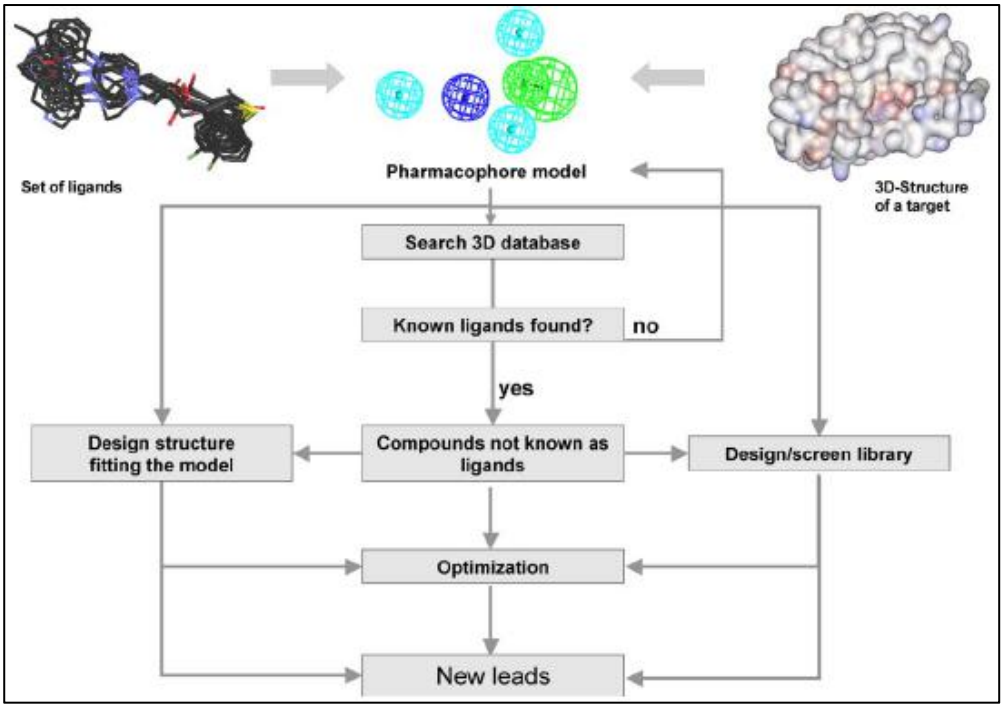


Figure 4 : Workflow of pharmacophore modelling from (Wolber & Langer.,2004)

Pharmacophore is defined as 3D arrangement of chemical groups common to active molecules and essential for their biological activities. Pharmacophore modelling is an important tool in drug discovery and in modern medicinal chemistry which was introduced by Ehrlich in 1909 to ease the understanding between the receptor-ligand interactions (Yang, 2010). Structure-based pharmacophore modelling is a strategy in the presences of a target

macromolecule to observe the interaction of proposed drug with the active site. Ligand-based drug pharmacophore modelling is a strategy to discover potential drug by generating a pharmacophore model using established drug to treat a diseases or infection without the involvement of a macromolecule (Radhakrishnan *et al*., 2017).

3.0 Materials and Method

The drug was designed by using pharmacophore modelling approach. Synthesis of compound was done at Natural Product Laboratory of Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS). Shimadzu Mass Spectrophotometer was used to record the molecular weight of the compounds. Characterization of the synthesized compounds was conducted by using Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). The Thin Layer Chromatography Technique (TLC) was used in this project to test the purity of the synthesized compounds. Stuart MP3 will be used to determine the melting point of each synthesized compound. The brine shrimp (*Artemia salina*) was used in the cytotoxicity assay.

3.1 Pharmacophore Modelling

Pharmacophore modelling was assisted by the computer software such as Ligandscout 4.3 and Chemsketch. This project was carried out by using Ligand-Based Drug Design (LBDD). The ligand-based drug design is based on mapping and orientation of certain features of a molecule that could form the basis for its high binding affinity and selectivity and also give information about functional group that bind to target and also type of interactions and atomic distance between functional group and interactions.

3.1.1 Pharmacophore Generation

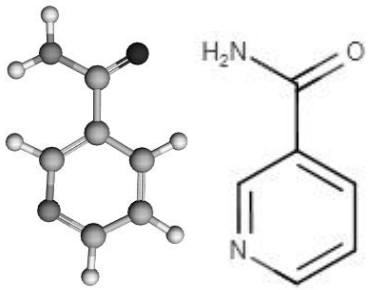
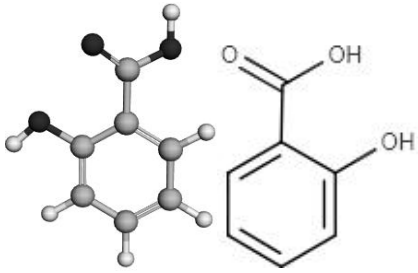
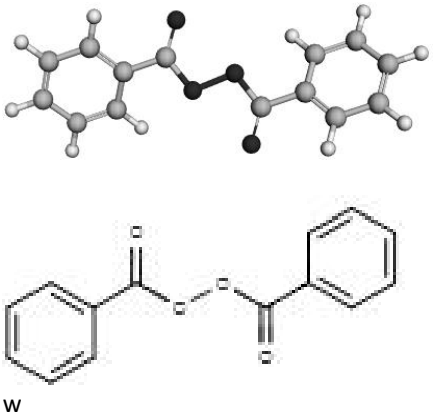
Ligand-Based pharmacophore modeling approach was used to evaluate the potential benzaldehyde derivatives compound by analysis using pharmacophore Fit-Value and screening based on the chemical features. To create the pharmacophore model, Ligandscout 4.3 computer software was used. LigandScout software efficiently generates 2D and 3D pharmacophore data set rapidly and transparently. Ligand-Based pharmacophore model was

created by alignment of all training set forming a set of model of established anti-acne drug. This model exhibited all the features from all of training set. The expected chemical features derived from the model using Ligandscout 4.3 software was Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), hydrophobic regions, and aromatic rings (Romli *et al*.,2017).

3.1.2 Training Set

This training set was a set of popular and effective anti-acne established drugs (Fox et al., 2016). The selection was essential for generating the pharmacophore model.

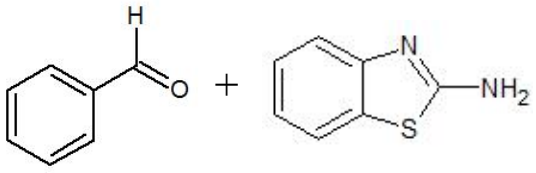
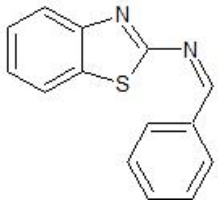
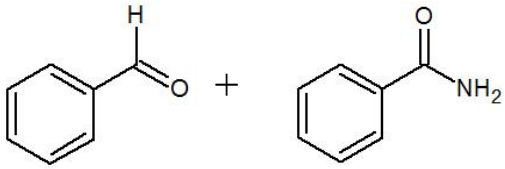
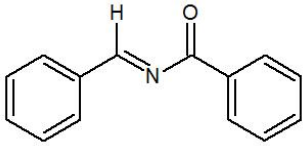
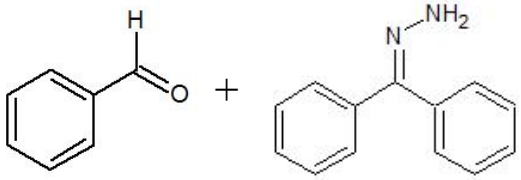
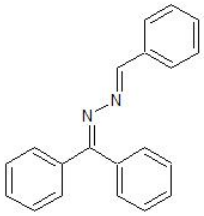
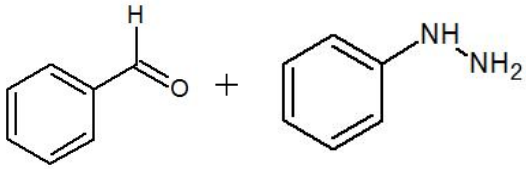
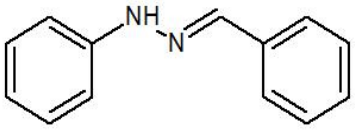
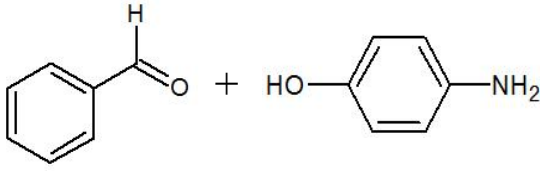
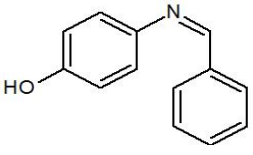
Table 1 Structure of selected training sets taken from https://www.drugbank.ca/

		
Nicotinamide	Salicylic Acid	Benzoyl Peroxide

3.1.3 Test Set

Test set was selected from the reaction of benzaldehyde and primary amine that will be synthesized. The 2D chemical structures of the benzaldehyde derivatives are drawn by using ChemSketch.

Table 2 List of Benzaldehyde Derivatives

Benzaldehye + Primary Amine	Reagent	Benzaldehyde Derivatives
 Benzaldehyde 2-aminobenzothiazole	EtOH Reflux 5hr	 (Z)-N-(1,3-benzothiazol-2-yl)-1-phenylmethanimine
 Benzaldehyde Benzamide	EtOH Reflux 5hr	 N-[(E)-phenylmethylidine] benzamide
 Benzaldehyde Benzophenone hydrazone	EtOH Reflux 5hr	 (1E)-1-benzylidene-2-(diphenylmethylidene) hydrazine
 Benzaldehyde Phenylhydrazine	EtOH Reflux 5hr	 (1E)-1-benzylidine-2-phenylhydrazine
 Benzaldehyde 4-aminophenol	EtOH Reflux 5hr	 4-[(Z)-benzylidineamino] phenol