Faculty of Resource Science and Technology

Synthesis and Characterisation of Halogenated Vanillin Derivatives as Potential Antibacterial Agents

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Synthesis and Characterisation of Halogenated Vanillin Derivatives as Potential Antibacterial Agents

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

The escalating issue of antimicrobial resistance has become a pressing global challenge and poses a significant threat to public health. Thus, new potent drugs derived from natural product are in demand in the pharmaceutical sector as it offers a wide range of chemical structures and diverse bioactive compounds. However, the extraction of secondary metabolites from natural resources are labour-intensive, time-consuming and frequently inefficient due to the mismatch between the active group and the target receptor. Chemical modification of a lead compound derived from natural resources such as vanillin has garnered increasing attention for pharmacological purposes. In this study, three different series of novel vanillin derivatives namely halogenated vanillin azo derivatives (**43a-h**) were successfully synthesised via diazo coupling reaction, while halogenated vanillin Schiff base derivatives were prepared via nucleophilic substitution reaction (**44a-h**). The hybrid molecules of vanillin azo-Schiff base derivatives bearing halogens (Br, Cl, F) (**45a-h**) were prepared from the reaction of **43a-h** with aniline to give significant yields of 67-83% via microwave irradiation method compared to the conventional heating (38-53%). The antibacterial assay of all synthesised compounds at a concentration of 100 ppm was evaluated employing Kirby-Bauer disc diffusion assays against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (S48/81). All target compounds were active against both bacterial strains in comparison to the parent vanillin. Compounds **43a-h** and **44a-h** were active against *S. aureus* with inhibition zones of 7-10 mm and 8-13 mm, respectively, but less active against *E. coli* with inhibition zones of 7-9 mm and 7-8 mm, respectively. Notably, compounds **45a-h** demonstrated good antibacterial activity (8-11mm) against both bacterial strains in comparison to the standard drug ampicillin (11 mm). Among the halogenated vanillin azo-Schiff base derivatives, compound bearing fluorine at *meta* position

(**45b**) demonstrated excellent antibacterial efficacy against *E. coli* and *S. aureus* with both inhibition zones of 11 mm respectively, comparable to standard drug ampicillin. The successful synthesis of these hybrid vanillin azo-Schiff base derivatives implies their potential utility in drug design that will aid in the development of new, potent antibacterial drugs with excellent properties.

Keywords: Vanillin, azo-Schiff base, *Escherichia coli, Staphylococcus aureus,* halogenated vanillin Schiff base

Sintesis dan Pencirian Terbitannya Vanillin Sebagai Agen Antibakteria Berpotensi

ABSTRAK

*Peningkatan isu ketahanan antimikrobial telah menjadi cabaran global yang mendesak dan menimbulkan ancaman yang ketara terhadap kesihatan awam. Oleh itu, penghasilan ubat-ubatan baru yang diperoleh daripada produk semula jadi mendapat permintaan dalam sektor farmaseutikal kerana menawarkan pelbagai struktur kimia dan sebatian bioaktif yang pelbagai. Walau bagaimanapun, pengekstrakan metabolit sekunder dari sumber semulajadi adalah intensif buruh, memakan masa dan sering tidak efisien disebabkan oleh ketidaksepadanan antara kumpulan aktif dan reseptor sasaran. Dalam kajian ini, tiga siri yang berbeza daripada terbitan vanilin yang baru iaitu terbitan azo vanilin berhalogen (**43a-h**) telah berjaya disintesis melalui tindak balas penggandingan diazo, manakala terbitan Schiff vanilin berhalogen juga telah disediakan melalui tindak balas penggantian nukleofilik (**44a-h**). Molekul hibrid terbitan vanilin azo-Schiff berhalogen (Br, Cl, F) (**45a-h**) telah disediakan melalui tindak balas **43a-h** dengan anilin untuk memberikan hasil yang signifikan, iaitu antara 67-83%, melalui kaedah radiasi gelombang mikro berbanding pemanasan konvensional (38-53%). Ujian antibakteria semua sebatian yang disintesis pada kepekatan 100 ppm telah dinilai dengan menggunakan ujian penyebaran cakera Kirby-Bauer terhadap* <u>Escherichia</u> <u>coli</u> *(ATCC 25922) dan* <u>Staphylococcus</u> <u>aureus</u> *(S48/81). Semua sebatian sasaran aktif terhadap kedua-dua strain bakteria berbanding vanilin induk. Sebatian **43a-h** dan **44a-h** aktif terhadap* <u>S.</u> <u>aureus</u> *dengan zon perencatan 7-10 mm dan 8-13 mm, secara berurutan, tetapi kurang aktif terhadap* <u>E.</u> <u>coli</u> *dengan zon perencatan 7-9 mm dan 7-8 mm, secara berurutan. Ternyata, sebatian **45a-h** menunjukkan aktiviti antibakteria yang baik (8-11 mm) terhadap kedua-dua strain bakteria berbanding ubat ampicillin standard (11 mm). Antara terbitan azo-Schiff vanilin berhalogen, sebatian yang* *mengandungi fluorin pada kedudukan meta (**45b**) menunjukkan keberkesanan antibakteria yang cemerlang terhadap* E. coli *dan* S. aureus *dengan zon perencatan yang sama sebanyak 11 mm, berbanding dengan ubat standard, ampisilin. Kejayaan sintesis terbitan hibrid vanilin azo-Schiff ini menggambarkan potensi penggunaan mereka dalam reka bentuk ubat yang akan membantu dalam pembangunan ubat antibakteria yang baru, berpotensi kuat dengan ciri-ciri yang unggul.*

Kata kunci: *Vanilin, azo-Schiff, Escherichia coli, Staphylococcus aureus, Schiff vanillin berhalogen*

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δ	Chemical shift in parts per million
V_{max}	Maximum vibrational frequency
CHN	Carbon, Hydrogen and Nitrogen
DMSO-d_6	Deuterated dimethyl sulfoxide
EtOH	Ethanol
FTIR	Fourier Transform Infrared
h	Hour
HCl	Hydrochloric acid
Hz	Hertz
J	Coupling constant in hertz
LB	Luria-Bertani
M	Molarity of solution
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
m.p.	Melting point
MW	Microwave irradiations
$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
rpm	Revolutions per million
TLC	Thin layer chromatography
UNIMAS	Universiti Malaysia Sarawak

CHAPTER 1

INTRODUCTION

1.1 Vanillin and its derivatives

Vanillin **1** (Figure 1.1) is an active metabolite with the IUPAC name of 4-hydroxy-3-methoxybenzaldehyde. It consists of active functional groups namely hydroxyl, methoxy, and carbonyl groups which are prone to multiple modifications and substitution reactions. Vanillin can dissolve in many environmentally benign solvents such as ethanol and water (Shakeel et al., 2015).

1

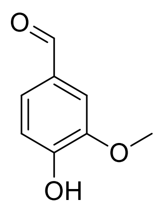


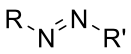
Figure 1.1: General Structure of Vanillin

Vanillin was originally obtained via extraction of vanilla orchids (*Vanilla planifolia*), and subsequently used widely as a flavoring agent in the food industry (Raymond Mohanraj et al., 2021) and pharmaceutical products (Anand et al., 2019). The extraction of vanillin as secondary metabolites from plants, however, is labor-intensive and lower in yield. In order to produce vanillin with high yield and more time effective, many synthetic pathways of vanillin have been introduced to reduce the production cost and increase the productivity of vanillin and its derivatives (Vijayalakshmi et al., 2019).

Synthetic vanillin offers increased accessibility for application in the food industry due to its cost-effectiveness compared to vanilla extract (Labuda, 2009). Furthermore, synthetic vanillin presents an intriguing avenue for extensive research in the realm of drug discovery, particularly in harnessing the potential of natural product-derived compounds (Kumar et al., 2012). One notable example is Trimethoprim, an antibiotic derived from vanillin, which exemplifies the efficacy of vanillin-based derivatives in addressing bacterial infections (Gulsia, 2020).

Many studies reported on the chemical modification of vanillin with improve biological activities such as antiproliferative (Birar et al., 2021), antibacterial (Pagariya et al., 2015; Raymond Mohanraj et al., 2021), antifungal, antimalarial, antioxidant (Narode et al., 2022), and anti-inflammatory (Boiko et al., 2019). Similarly, chemical modification of an active scaffold from other natural resources such as aspirin (Nordin et al., 2020) and coumarin (Mortadza & Ngaini, 2023) has gained interest nowadays due to its known properties and able to reduce the adverse effects (Cava-Roda et al., 2021). The active functional group in vanillin is susceptible for the formation of other active moieties containing nitrogen such as azo dyes (N=N) or imine group (HC=N) due to its available functional groups (Pagariya et al., 2015). Nitrogen is one of the important atoms in natural product moieties. Alkaloids, cytokinin, proteins, peptides, DNA, and RNA are a few examples of biologically significant secondary metabolites that contain nitrogen atoms (Elshafie et al., 2023). These nitrogen atoms can also be found abundantly in organic compounds such as caffeine (Faudone et al., 2021), melamine (Maleki et al., 2018), urea (Pradana et al., 2021) and dietary protein (Lassaletta et al., 2016). In pharmaceutical industry, over 75% of drugs with structural frameworks incorporating nitrogen-containing heterocyclic moieties have received FDA approval and are commercially available in the market (Kerru et al., 2020). Nitrogen atoms are frequently formed as constituents of important atomic centers for intra- and intermolecular interactions (Desiraju, 2000). The incorporation of active moieties into the vanillin network via active functional group is able to improve biological properties (Chigurupati, 2015; Pagariya et al., 2015). This remarkable biological activity has raised interest among researchers and are extensively studied.

Azo dyes are examples of compounds containing nitrogen to nitrogen double bond (N=N) as their chromophores. The active N=N chromophore has offered wide spectrum of biological properties (Mezgebe & Mulugeta, 2022). The dyes depicted in Figure 1.2 consist of a functional group R-N=N-R', wherein R and R' denote aryl or alkyl groups. Two aryl groups produce the simplest type of azo derivatives and are easily prepared via an azo-coupling reaction involving two aromatic rings joined together by an active -N=N- azo moiety (Maliyappa et al., 2022). This azo moiety can form mono- or polycyclic compounds by conjugating with either different or identical aromatic rings (Benkhaya et al., 2020).



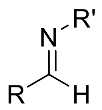
2

R/R': Alkyl or aryl group

Figure 1.2: General Structure of Azo Dyes

The active aryl/alkyl and -N=N- groups can form good interaction with receptors for a remarkable biological property. In acidic conditions, the N=N moiety is easily protonated, which improves the biological properties of the compound (Abd Halim & Ngaini, 2017). Beside pharmaceutical application, numerous applications of azo dyes can be found in textiles (Ravi et al., 2021), foods, paints, and cosmetics (Ngaini & Mortadza, 2019).

Similar to azo dyes, Schiff base is another type of compounds containing nitrogen atom as an active chromophore (Raczuk et al., 2022). It possesses a distinctive structure consisting of a nitrogen atom bonded to a carbon atom via a double bond (-C=N-), where R and R’ typically an aryl or alkyl group (Kajal et al., 2013). Schiff bases are commonly formed by the condensation reaction between a primary amine and a carbonyl compound, such as an aldehyde or a ketone.



R/R': Alkyl or aryl group

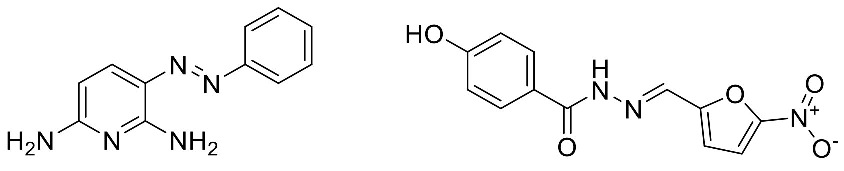
3

Figure 1.3: General Structure of a Schiff Base

Due to their imine or azomethine (-C=N-) functional group, Schiff bases are flexible compounds that have grown in significance in the design and development of numerous bioactive lead compounds (Kajal et al., 2013). Schiff bases display beneficial biological features such anti-inflammatory (Kajal et al., 2013), antibacterial (Chigurupati, 2015), anticancer (Suyambulingam et al., 2020), antioxidant (Vhanale et al., 2022), and anti-schistosomiasis (Scarim & Chin, 2021). Schiff bases are also used as ligands to complex metal ions and have a variety of uses, including as pigments and dyes (Shi et al., 2009; Siddiqui et al., 2006).

1.2 Problem statement

In recent years, scientists have been looking for an effective drug to lessen the grave health threat that pathogenic bacterial resistance poses to humans. Due to the rising costs in drug development process, finding a new and effective drug has become challenging to researchers (Ngaini & Mortadza, 2019). Natural product-based compounds such as penicillin (Kaur et al., 2020), coumarin (Ngaini & Mortadza, 2019) and aspirin (Nordin et al., 2020) have been used in traditional medicine to treat pain and fever. Nevertheless, the extraction of natural products is time-consuming, costly and lower in yield. Many synthetic approaches via chemical modification of natural product-based molecules with an active scaffold has become the advantage to enhance the effectiveness of the compounds and increases the likelihood of new interactions of the potential drugs with the biological targets (Ngaini & Mortadza, 2019).



4 5

Figure 1.4: Chemical Structure of Phenazopyridine **4** and Nifuroxazide **5**

Based on the literature precedence, a variety of drugs containing nitrogen chromophores (e.g. N=N moieties) (Figure 1.4) that are commercially available such as phenazopyridine **4** for urinary tract analgesia (Adu et al., 2020). Similarly, nifuroxazide **5** is another commercially available drug derived from Schiff base (CH=N) and clinically proven to treat respiratory tract infections (Hassan et al., 2015). Most of the current studies reported on the potential drugs derived from natural resources are focusing on either azo dyes or Schiff bases. However, less research discussed the possibility of azo dyes and Schiff bases coexisting in a single molecule. Incorporation of various active pharmacophores into one active scaffold of natural product can increase its biological activities by interaction with multiple targets with stronger binding affinity. It is envisaged that the synergistic interactions of natural product such as vanillin incorporated with azo and Schiff base moieties and other active substituents such as halogens can form stronger binding interaction with the biological target and envisage to improve the biological activity of the compound.

1.3 Objectives

The research embarked on the following objectives:

i. To synthesise halogenated vanillin derivatives bearing azo (N=N) **(43a-h)**, Schiff base (C=N) (**44a-h**) and halogenated azo-Schiff base vanillin (**45a-h**) bearing Br, Cl, F at *ortho*, *meta*, and *para* position.

ii. To study the CHN, FTIR, ^{1}H NMR and ^{13}C NMR spectroscopies of the halogenated vanillin derivatives (**43a-h, 44a-h** and **45a-h**); and

iii. To evaluate the antibacterial activity of the halogenated vanillin derivatives (**43a-h, 44a-h** and **45a-h**) against *E. coli* (ATCC 25922) and *S. aureus* (S48/81).

CHAPTER 2

LITERATURE REVIEW

2.1 Vanillin and its background

Vanilla is a common natural essence and culinary prominence that is derived from the beans of the *Vanilla planifolia* orchid (Gulsia, 2020). *Vanilla planifolia* and *Vanilla tahitensis* are two popular species out of 110 other species that are being cultivated and allowed to be used in food (Havkin-Frenkel & Belanger, 2007). The vanilla orchid which was originally native to Mexico boasts a widespread presence in various regions across the globe including Madagascar, Indonesia, Tahiti and Tonga (Jadhav et al., 2009). Vanilla beans continue to captivate the palates of individuals with an annual global demand surpassing 2,000 tons on average (Ciriminna et al., 2019; Havkin-Frenkel & Belanger, 2007).

Vanillin is a vital organic constituent obtained from vanilla beans with the yield of 1-2% (Anand et al., 2019). Vanillin was first isolated by Gobley in 1858 from *Vanilla planifolia* pods (Ciriminna et al., 2019). It can be extracted using conventional methods such as solvent extraction and supercritical liquid extraction (Jadhav et al., 2009). Vanillin can also be produced from the hydrolysis of glucosides presence in various oils, balsams, resins and woods (Jenkins & Erraguntla, 2014).

Vanillin has an IUPAC name of 4-hydroxy-3-methoxybenzaldehyde (Jenkins & Erraguntla, 2014). It has a low molecular weight of 152.15 g/mol with a boiling point of 285°C and melting point of 80–83 °C (Kumar et al., 2012). It presents as a white needle-like crystalline with a distinctively sweet and persistent vanilla-like aroma (Gulsia, 2020). Similar to other low-molecular-weight phenolic compounds, the active groups in the vanillin