

Synthesis and Biological Activity of Ampicillin Derivatives

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(23107)

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Declaration

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning. I hereby declare that this project is the work of my own excluded for the references document and summaries that have been acknowledge.

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List of abbreviations

Minimum Inhibition concentration	MIC
Millimeter	mm
Mili Liter	mL
Mili Mole	mmol/10 ⁻³
Microgram	μg
Fourier Transform Infrared Spectroscopy	FTIR
Nuclear Magnetic Resonance	NMR
Thin Layer Chromatography	TLC
Carbon Hydrogen Nitrogen analysis	CHN analysis

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ABSTRACT

Ampicillin is a beta-lactam antibiotic that has been widely used clinically to treat bacterial infections particularly pathogens such as *Escherichia coli*. This research focused on the synthesis of ampicillin derivatives by covalently linking its amine bond through condensation reaction to isomers of hydroxybenzaldehyde (2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, and 4-hydroxybenzaldehyde) as well as 2,4-dimethoxybenzaldehyde. Five ampicillin derivatives compound have been synthesized. The synthesized compounds were characterized using FTIR, ¹HNMR and ¹³CNMR. The antibacterial activity was evaluated according to its zone of inhibition and minimum concentration value using turbidimetric method against the growth of *Escherichia coli*. However, the results indicated that the synthesized compound did not exhibit any significant antibacterial activity against *Escherichia coli*. The effects of the structure of the investigated compounds on the antibacterial activity were discussed.

Keywords: Ampicillin, Schiff base, Antibacterial activity, Hydroxybenzaldehyde

ABSTRAK

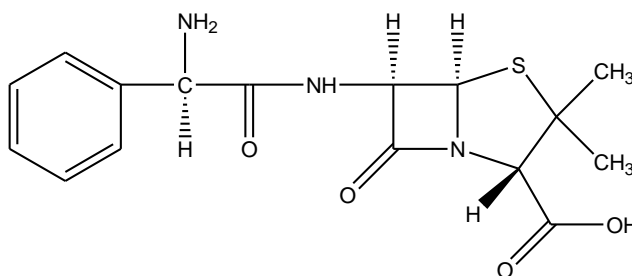
Ampicillin adalah antibiotik beta-lactam yang telah digunakan secara meluas untuk merawat jangkitan bakteria khususnya patogen seperti Escherichia coli. Kajian ini memberi tumpuan kepada sintesis derivatif ampicillin melalui ikatan kovalen yang menghubungkan kumpulan berfungsi amida dengan isomer-isomer hydroxybenzaldehyde (2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde) dan juga 2,4-dimethoxybenzaldehyde melalui tindak balas kondensasi. Lima jenis sebatian derivatif ampicillin telah disintesis. Sebatian yang disintesis telah dicirikan melalui penggunaan FTIR, ¹HNMR, dan ¹³CNM. Aktiviti bakteria telah dinilai mengikut zon perencatan dan nilai kepekatan minimum dengan menggunakan kaedah turbidimetric terhadap pertumbuhan Escherichia coli. Walau bagaimanapun, keputusan menunjukkan bahawa sebatian yang disintesis tidak mempamerkan apa-apa aktiviti antibakteria terhadap Escherichia coli. Kesan struktur sebatian atas aktiviti antibakteria telah disiasat dan dibincangkan.

Kata kunci: Ampicillin, Schiff base, Antibacterial activity, Hydroxybenzaldehyde

Chapter 1

Introduction

Ampicillin **1** is a beta-lactam antibiotic that has been used extensively to treat bacterial infections since 1961. It acts as a bactericidal broad-spectrum penicillin belonging to the group of aminopenicillins with the formula, $C_{16}H_{19}N_3O_4S$ and a molecular weight of $349.41\text{g}\cdot\text{mol}^{-1}$. Chemically, it is known as 6-([2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. The melting point of ampicillin is $208\text{ }^{\circ}\text{C}$ and it appears as a white to off white crystalline powder.



1

Figure 1: Structure of ampicillin, **1**

Ampicillin **1** has a broad spectrum of action which extends to both Gram-positive and Gram-negative organisms by suppressing its cell wall synthesis (Katzung, 2001). Similar in action to benzylpenicillin, ampicillin is more stable in stomach acids and therefore may be given orally and it is also more active against certain strains of bacteria. It is used regularly to treat common urinary-tract infections, some respiratory infections, and bacterial meningitis in children (Drug Information Portal 2001).

Some of the clinically relevant pathogens covered by ampicillin are *Escherichia coli*, *Proteus mirabilis*, *Salmonellae typhi*, *Shigellae dysenteriae*, *Haemophilus influenza* and *Bordetella pertussis*. However, all *Pseudomonas* and most strains of *Klebsiella* and *Aerobacter* are considered resistant to ampicillin (Chambers, 2001). The potential side effects of ampicillin are similar to those of other penicillins, mainly allergic reactions ranging from skin rashes and hives to life-threatening anaphylactic shock (Drugs Information online 2011).

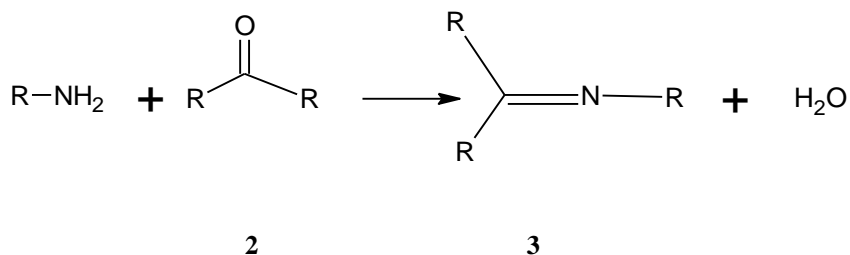
People who are allergic to other drugs in this family are also likely to react to ampicillin. The incidence of skin rashes is higher with ampicillin than with other penicillins, a factor that suggests a possible toxic reaction, as well as a truly allergic response. There is an increase in the mortality rate associated with infectious diseases which are directly related to bacteria that exhibit multiple resistances to antibiotics. For ampicillin, a complete resistance has been established for *Bacteroides fragilis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Proteus rettgeri* und *morganii*, *Pseudomonas aeuginosa* and *Serratia narcescens* (Chow, 1991).

A number of ampicillin derivatives have been synthesized to improve its pharmacological properties. The primary amine group in ampicillin is capable of reacting with substituted aromatic aldehyde or ketone to form Schiff bases. Schiff bases are known to exhibit a broad range of biological activities particularly as an antimicrobial agent (Przybylski *et al.*, 2009). The imine group present in Schiff bases has been shown to be critical to their biological activities (Guo *et al.*, 2007). In recent years, ampicillin derivatives have been reported to exhibit antibacterial activity such as those against all pyocin types of *Pseudomonas aeruginosa* strains which are highly resistant to ampicillin (Chen *et al.*, 1997).

In 1999, Tsou and co-workers studied on the antibacterial activities of ampicilin derivatives. Ampicilin are derived by covalently linking its amide bond to a fluoroquinolone (Tsou *et al.*, 1999). Various substituents on the aromatic bearing aldehyde of the reacting material can be modified to alter the Schiff bases biological properties. A report has also shown that halogen substituted Schiff bases exhibited better antimicrobial activity than ampicillin alone (Junne *et al.*, 2010). In addition to that, there were other reports on ampicilin reacting with other compounds such as sulbactams (Hartmut M, 2008) and flavones (Unlusoy *et al.*, 2004).

Schiff base

Schiff bases **3**, named after Hugel Schiff is formed by condensation of aldehyde **2** or ketone **2** with a primary amine according to the scheme 1:



Scheme 1: General reaction of Schiff base formation

It is structurally a nitrogen analogue of an aldehyde or ketone in which the carbonyl group C=O has been replaced by an imine or azomethine group. Schiff bases are the important compound owing to their wide range of biological activities and industrial application (Wang *et al.*, 2008). They have been found to possess the pharmacological activities such as antimalarial (Li *et al.*, 2003) anticancer (Villar *et al.*, 2004) antibacterial (Venugopal and Jayashree, 2008) antifungal (Pandey *et al.*, 2003) antitubercular (Bhat *et al.*, 2005), anti-inflammatory and

antimicrobial (Wadher *et al.*, 2009). They also serve as a back bone for the synthesis of various heterocyclic compounds.

The presence of azomethines, HC=N is responsible for antimicrobial activity, which can be altered depending upon the type of substituent present on the aromatic rings (Kumar *et al.*, 2010). In the formation of ampicillin derivative, the primary amine group of ampicillin reacts with an aromatic aldehyde leading to the replacement of its carbonyl group by an imine group. Derivatives of Schiff bases have been synthesized previously and its antimicrobial potential towards various human pathogenic bacteria bears some positivity (da Silva *et al.*, 2011).

1.1 Problem statement

Due to the increasing multiple resistances towards antibiotics, ampicillin is still somewhat a problem in the medical field. The prevalence of antibiotic resistant bacteria towards ampicillin is the result of prolong duration of therapeutic exposure. Ampicillin shows complete resistance to certain microorganism such as *Klebsiella sp*, *Enterobacter sp*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and no activity is recorded against beta-lactamase producing *Staphylococci*. Pathogens such as *Streptococcus pyogenes*, *Bacillus subtilis*, *Esherichia coli* and *Salmonella typhi* known to be susceptible to ampicillin treatment still show lower minimum inhibition concentration when compared to other prevailing antibiotics from fluoroquinolones class such as ofloxacin (Chen *et al.*, 1997).

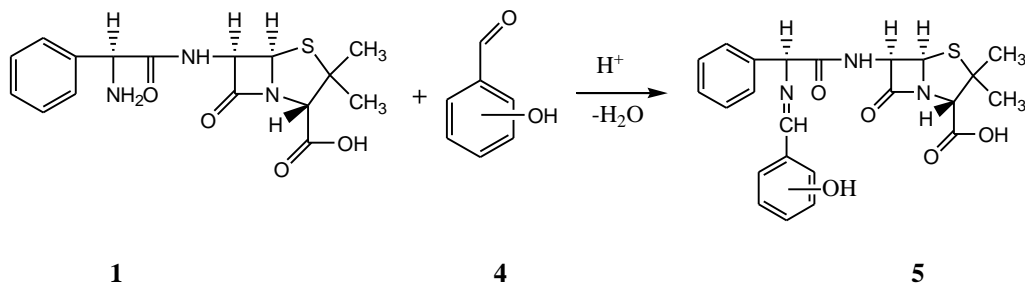
Reports showed that ampicillin derivatives exhibit improved antibacterial action towards susceptible bacterias and some effects towards highly resistance ones (da Silva *et al.*, 2011). However, there is still an urgent need for new antibacterial agents to be synthesized in order to overcome the problem of bacterial resistances. In addition to that, very minimal researches have been reported on the synthesis of ampicillin Schiff base derivatives. Hence, more studies on the antibacterial capabilities of ampicillin Schiff bases should be conducted. For this reason, this project was proposed to prepare ampicillin derivatives, by reacting ampicillin with isomers of hydroxybenzaldehyde and chlorobenzaldehyde. The presence of imine group in ampicillin is shown to be critical in their antibacterial activity (Zheng *et al.*, 2009). Different isomers of hydroxybenzaldehyde and halogen substituted benzaldehyde was investigated for antibacterial activity.

1.2 Objectives

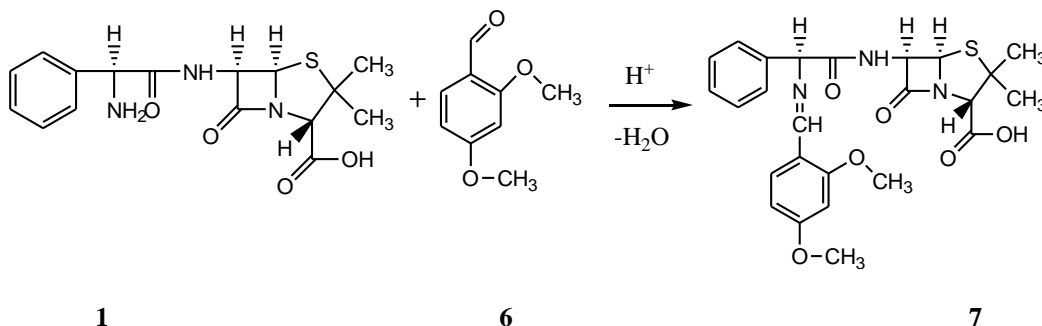
The main objectives of the project are:

- 1.) To synthesize ampicillin derivatives using series of aldehydes
- 2.) To characterize the synthesized ampicillin derivatives derived from ampicillin and series of aldehydes using Infra-Red (IR), ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy.
- 3.) To investigate the antibacterial activities of synthesized ampicillin derivatives against *Escherichia coli*

The synthesis of ampicillin derivatives **5** & **7** from series of aldehydes is shown in Scheme 2 & 3:



Scheme 2: Synthesis of ampicillin derivatives, **5** from isomers of hydroxybenzaldehyde, **4**



Scheme 3: Synthesis of ampicillin derivatives, **7** from 2,4-dimethoxybenzaldehyde, **6**

Chapter 2

Literature review

2.1 Ampicillin

Ampicillin, a beta-lactam antibiotic is used to treat many different types of infections caused by bacteria, such as ear infections, bladder infections, upper respiratory tract infections, genital infections, and *Escherichia coli* or *salmonella* infections. It is classified as a bacteriicidal broad- spectrum penicillin belonging to the group aminopenicillins (Chambers, 2001). Penicillin therapies had only been effective against Gram-positive organisms such as *staphylococci sp* and *streptococci sp*, while ampicillin has a broad spectrum of antimicrobial action towards both Gram-positive and Gram-negative organisms (Drug Information Portal 2011). The amine group of ampicillin helps the drug by penetrating the outer membrane of gram-negative bacteria. Ampicillin works by acting as a competitive inhibitor of the enzyme transpeptidase by inhibiting the third and final stage of bacteria cell wall synthesis in binary fission (Kasten and Reski, 1997).

Clinically relevant bacterias covered by ampicillin includes *Escherichia coli*, *Proteus mirabilis*, *Salmonellae typhi*, *Shigellae dysenteriae*, *Haemophilus influenza* and *Bordetella pertussis*. In contrast to that, all *Pseudomonas sp* and most strains of *Klebsiella sp* and *aerobacter sp* are considered to show high resistance (Chambers, 2006). Ampicillin are the most active oral *beta-lactam* antibiotics against penicillin resistant *pneumococci* and are the preferred beta-lactam antibiotics for treating infections suspected to be caused by these resistant strains. Ampicillin is also useful for treating serious infections caused by penicillin-susceptible organisms, including anaerobes, *enterococcus sp*, and susceptible strains of Gram-negative cocci and bacilli such as *Escherichia coli*, *Haemophilus influenza*, and *salmonella* species (Arthur and Courvalin, 1993).

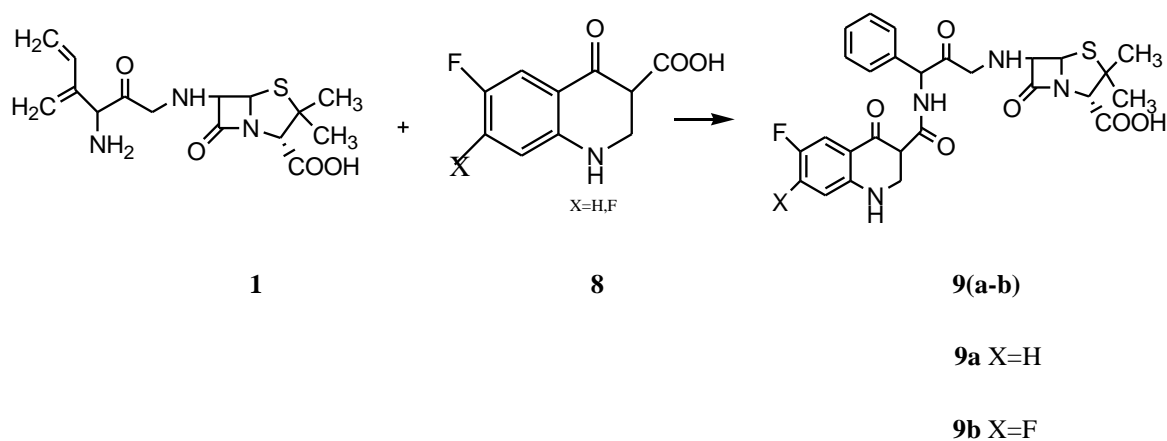
Unfortunately, many strains of the Gram-negative species that were uniformly susceptible now produce beta-lactamases and are therefore resistant to ampicillin, precluding its use for empirical therapy of urinary tract infections, meningitis, and typhoid fever (*Emery CL et al.*, 1997). Beta-lactamases are enzymes produced by some bacteria and are responsible for their resistance to beta-lactam antibiotics like penicillins (Jacoby and Luisa, 2005). There are a few multidrug resistance mechanisms attained by these microorganisms such as enzymatic deactivation of antibiotics, alteration of antibiotics target sites as well as decreasing cell wall permeability to antibiotics (Stix, 2006).

In addition to that, ampicillin is also not active against *klebsiella sp*, *enterobacter sp*, *pseudomonas sp*, *citrobacter sp*, and other Gram-negative aerobes that are commonly encountered in hospital acquired infections (Chow, 1991). Although several classes of antibacterial agents are presently available, resistance in most of the pathogenic bacteria to these drugs constantly emerges. In order to prevent this serious medical problem, the elaboration of new types of antibacterial agents or the expansion of bioactivity of the previous drugs is a vital task.

The increasing resistances towards antibiotic have been found to increase the mortality rate associated with infectious diseases related to resistant bacteria. The prevalence of antibiotic resistant bacteria is a result of the increasing duration of exposure and excessive use to antibiotics (Miranda *et al.*, 1996). As resistance towards antibiotics becomes more prevalent, the search for better antimicrobial agents are currently the major interests among researchers. Therefore, numeral ampicillin derivatives have been synthesized to meet this demand (Lin *et al.*, 2003).

2.1.1 Antibacterial activities of ampicillin derivatives

In 2003, Lin and co-workers reported on the synthesis of fluoroquinolonyl ampicillin derivatives. Compounds of fluoroquinolonyl ampicillin derivatives, **9a-b** can be prepared by reacting ampicillin, **1** with fluoroquinolone, **8** as shown in scheme 4.



Scheme 4: Synthesis of *N*-fluoroquinolonyl ampicillin derivatives, **9a-b**

Ampicillin and fluoroquinolones are antibacterial agents known for its potent action against a broad spectrum of bacterial species. The ampicillin derivatives compound are formed by covalently linking the amide bond of ampicillin to a halogen substituted fluoroquinolone, **8** to form Schiff base derivatives, **9a-b**.

A wide range of Gram-positive and Gram-negative bacterial species such as *Escherichia coli* and *Staphylococcus aureus* were used in the study. Both bacterial species are known to be susceptible towards ampicillin. Compounds, **9a** and **9b** are reported to exhibit antibacterial activity against all pyocin types of *Pseudomonas aeruginosa* strains highly resistant to ampicillin. This milestone may be explained by how different positions of halogen substituent on the fluoroquinolone compound influences the antibacterial activity (Lin *et al.*, 2003). Highly

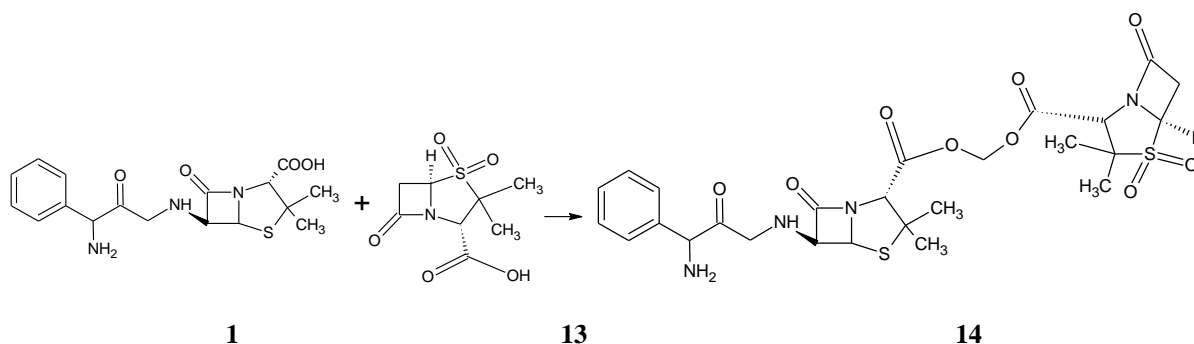
resistance bacterial species such as *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were also tested against the ampicillin derivatives.

Compounds **9a** and **9b** exhibited similar MIC (minimum inhibition concentration) value of 0.25µg/ml when tested against *Staphylococcus aureus*, indicating an improved effectiveness when compared to ampicillin itself with MIC of 0.5µg/ml. As for *Escherichia coli*, only compound **9b** showed improved effectiveness with MIC value of 1µg/ml compared to ampicillin alone with MIC of 2µg/ml. The lowered MIC value shown by both ampicillin derivatives compound supports the fact that Schiff bases formed through amine substitution provides better antibacterial effects than ampicillin acting alone.

A more significant discovery were found when compound **9a** and **9b** exhibited a MIC value below 16µg/ml when tested against highly resistant bacterial species such as *Salmonella typhi*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. This effectiveness is rendered significant due to the fact that ampicillin showed MIC value above 128µg/ml against the three previously mentioned bacterial species. This discovery of potentially effective ampicillin derivatives provides pathway for the manipulation of ampicillin functional groups such as the amine group. This enables the amine group to form biologically active Schiff bases derivatives through modification of aromatic aldehyde substituents.

In another research concerning ampicillin derivatives, in 2004, Unlusoy and co-workers reported on the synthesis and antimicrobial activities of some flavonyl Pro-drug esters of ampicillin as shown in Scheme 5. A pro-drug is a pharmacological substance that is administered in its inactive form. Upon administration, the pro-drug is metabolised into an active metabolite, a process known as bioactivation (Wu, 2009). Flavonyl Pro-drug esters of ampicillin are formed

Besides flavonyl pro-drug esters of ampicillin, ampicillin can also be combined with other pharmaceutical compound such as sulbactam. Sulbactam is a compound given in combination with ampicillin to inhibit beta-lactamase, an enzyme produced by bacteria that destroys antibiotics (TotirMA *et al.*, 2007). Sulbactam along with ampicillin forms a prominent antibiotic called Sultamicillin or commonly known as Unasyn®. Sultamicillin is widely used in treating infections caused by bacteria resistant to beta-lactam antibiotics (Singh, 2004) and known to be active against a wide range of bacterial groups, including *Staphylococcus aureus*, *Enterobacteriaceae*, and anaerobic bacteria. However it is not active against *Pseudomonas aeruginosa* (Chambers, 2001). Sultamicillin compound is formed when ampicillin is reacted with sulbactam as shown in Scheme 6.



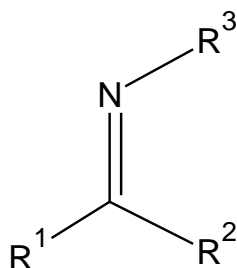
Scheme 6: Synthesis of Sultamicillin compound, **14**

Studies have shown that synthesis of sultamicillin, **14** involves *O*- alkylation of ampicillin itself or *N*-protected ampicillin with a halomethyl ester of sulbactam (del Pozo *et al.*, 2001). In addition, del Pozo *et al.*, (2001) also mentioned the use of imines in the sultamicillin field to protect the nitrogen of ampicillin during the coupling reaction with sulbactam. The imines were produced in good yields whilst exhibiting high stability under conditions of coupling process. Imines were deemed as an important intermediate for efficient synthesis of sultamicillin. On top of that, a susceptibility studies done in vitro have demonstrated that sultamicillin has better

antimicrobial activity than ampicilin when used against ampicilin resistant bacterias. (Hartmut M, 2008).

2.2 Schiff Bases

Schiff bases are formed when any primary amines reacts with an aldehyde or ketone under specific conditions. A Schiff base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by a C=N-R group. It is usually formed by condensation of an aldehyde or ketone with a primary amine.



$R^1, R^2, R^3 = \text{alkyl or aryl}$

4

Figure 2: General structure of a Schiff base, 4

The formation of Schiff base from aldehyde and ketone is acid catalyzed and the reaction type is nucleophilic addition of the amine to the carbonyl compound, followed by transfer of a proton from nitrogen to oxygen to a stable carbinolamine (McMurray, 2005). Schiff bases are known to be promising antibacterial agents and are extensively used in medical practices. Bacteria have been shown to exhibit multiple resistances to antibiotics, hence causing an increase in the mortality rate associated with infectious diseases. The lack of effective treatment is the main cause of this problem (Baquero, 1997). The development of new ampicillin Schiff bases with

novel and more efficient mechanism of action is definitely an urgent medicinal need (Louis B, 2006).

2.2.1 Preparation of Schiff base

The first preparation of imines was reported in the 19th century by Schiff and since then a variety of methods for the synthesis of imines have been described (Zheng *et al.*, 2009). The efficiency of these methods is dependent on the use of highly electrophilic carbonyl compounds and strongly nucleophilic amines (Chakraborti AK *et al.*, 2004).

In 2005, Pannerselvann and co-workers proposed as an alternative the use of substances that function as Bronsted-Lowry or Lewis acids to activate the carbonyl group of aldehydes, catalyze the nucleophilic attack by amines, and dehydrate the system, eliminating water as the final step. Examples of Bronsted-Lowry or Lewis acids used for the synthesis of Schiff base include ZnCl₂, TiCl₄, H₂SO₄ and HCl (Panneerselvann *et al.*, 2005).

In the past 12 years, a number of innovations and new techniques have been reported, including solvent-free/clay/microwave irradiation, solid –state synthesis, K-10/microwave, water suspension medium, solvent-free/CaO/microwave and silica/ultrasound irradiation (Vazquez, 2004). Among these innovations, microwave irradiation has been extensively used due to its operational simplicity, enhanced reaction rates, and great selectivity (Gopalakrishnan *et al.*, 2007).

Presently, the conventional way of synthesizing Schiff base involves condensation reaction of an aldehyde with amine in alcohol. The reaction is simple and can be conducted under open air condition. Formation of Schiff base is also favored in presence of a dehydrating agent such as MgSO₄ for water is the by-product of the reaction (Chakraborti AK *et al.*, 2004).