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Research Article

Characterization of ^{1}H NMR and ^{13}C NMR Spectrometry and Antibacterial Activities of 4-*tert*-Butylbenzoyl Amoxicillin

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ABSTRACT

The synthesis of 4-tert-butylbenzoyl amoxicillin has been done by reacting amoxicillin with 4-tert-butylbenzoyl chloride. The product was characterized using proton and carbon-13 spectrometry. Antibacterial activity of 4-tert-butylbenzoyl amoxicillin against *Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus* was tested using paper disk diffusion method. Results showed that 4-tert-butylbenzoyl amoxicillin had a specific spectrum activity. Antibacterial test of 4-tert-butylbenzoyl amoxicillin showed clear zones around the disc paper for *Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus.* The study results indicated 4-tert-butylbenzoyl amoxicillin has antimicrobial properties but is categorized as a less-effective antibiotic agent based on the response to bacterial growth inhibition.

INTRODUCTION

Pathogenic enteric bacteria are a common bacteria that infect the digestive tract of both animals and humans. Many of these bacteria come from contaminated food and water. These bacteria are a group of gram-negative and gram-positive rods that are widely bred in clinical laboratories and most commonly cause gastrointestinal diseases. Families including pathogenic enteric bacteria that frequently contaminate food include several genera, including *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Bacillus subtilis* (*B. subtilis*) (Siddiq *et al*., 2018).

Infectious disease is a threat to human survival and has become the third leading cause of death in the world (Dzen *et al*., 2003). Prevention of infectious diseases is generally done by administering antibiotics. Inappropriate use of antibiotics such as inaccurate indications of use, free use by the public, as well as inappropriate dosage and duration of administration will cause new problems, namely increased bacterial resistance to antibiotics (Dzen *et al*., 2003). The incidence of bacterial resistance continues to increase in various parts of the world. However, the increase was accompanied by a downward trend in the development of new antibiotics (Buntaran, 2007; Finch and Hunter, 2006). To overcome this problem, efforts are needed to develop new antibiotics (Spellberg *et al*., 2004; Yoneyama *et al.,* 2006).

Amoxicillin is a penicillin derivative whose structure is similar to ampicillin, with the difference in the presence of a hydroxy group at the position of the benzene rings. Some of the benefits of amoxicillin compared to ampicillin is the absorption of the drug in the digestive tract that is more complete so that plasma levels are higher. Maximum blood levels are achieved within 1 hour after oral application (Soekardjo and Siswandono, 2000). On the one hand, amoxicillin is not effective against some bacteria including *E. coli* (Krisnaningsih *et al*., 2005) and *S. aureus* (Shituu *et al*., 2011). Therefore, modification of amoxicillin compounds is needed that can improve its performance or reduce antibiotic resistance in bacteria.

On the other hand, 4-*tert*-butylbenzoyl amoxicillin compound has been synthesized and its physical and chemical properties

characterized including organoleptic, pH, melting point, Rf value on thin layer chromatography, maximum wavelength on UV-Vis spectrophotometry, and functional groups using IR spectrophotometry (Siddiq *et al.*, 2018).

In this research, further characterization was done in the form of structural characterization using proton and carbon-13 spectrometry. Then, the antibacterial activity was tested against *E. coli, S. aureus*, and *B. subtilis*.

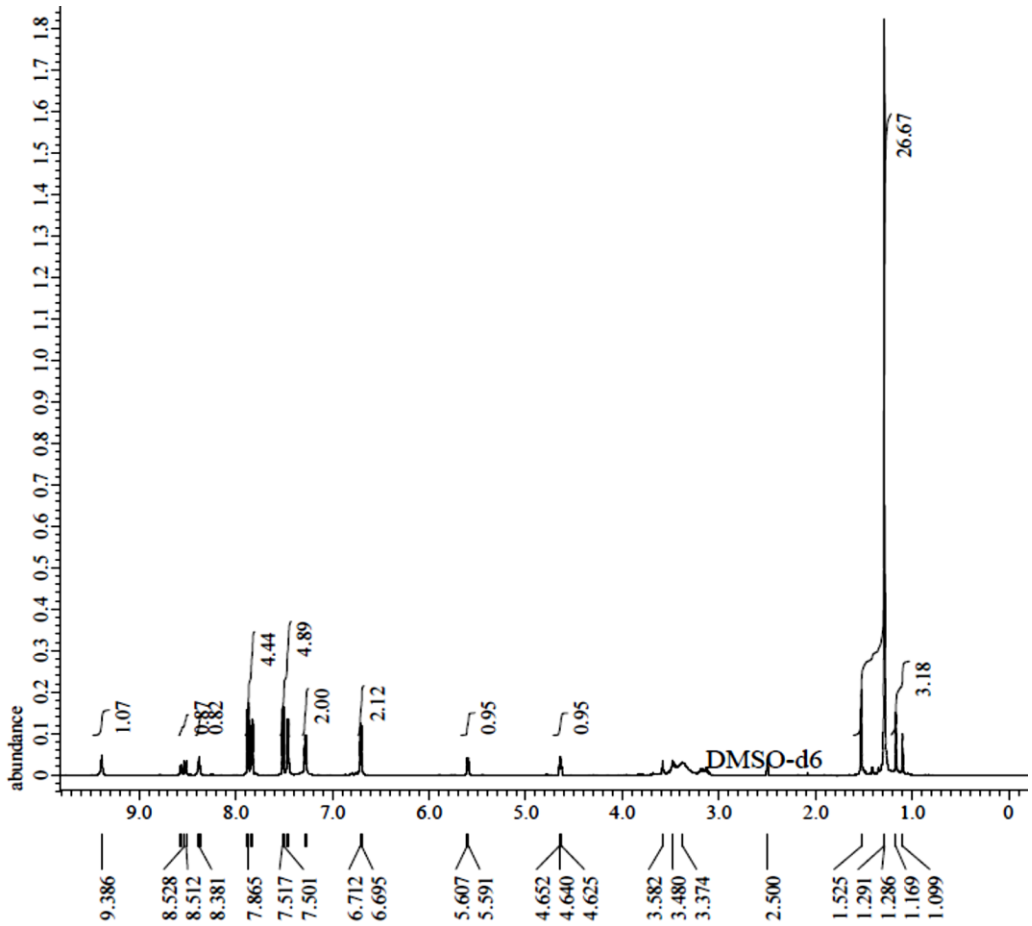


Figure 1. ^{1}H NMR spectrum of 4-*tert*-butylbenzoyl amoxicillin compound.

Table 1. ^{1}H NMR 4-*tert*-butylbenzoyl amoxicillin data, chemical shift, multiplicity, H amount, and Group

Chemical Shift (ppm)	Multiplicity	H amount	Group
1,099	s	3	-C**H**$_3$
1,169	s	3	-C**H**$_3$
1,286	d	2	-C**H**$_2$-
1,525	s	1	-C**H**-
4,640	t	1	R-CO-O-C**H**-
5,607	d	1	R-CO-N**H**-
6,695	d	2	Ar-C**H**
7,269	d	2	Ar-**H**
7,501	q	3	Ar-**H**
7,865	q	3	Ar-**H**
8,381	t	2	R-CO-N**H**$_2$-
8,528	d	1	R-CO-N**H**-
9,386	s	1	R-COO**H**

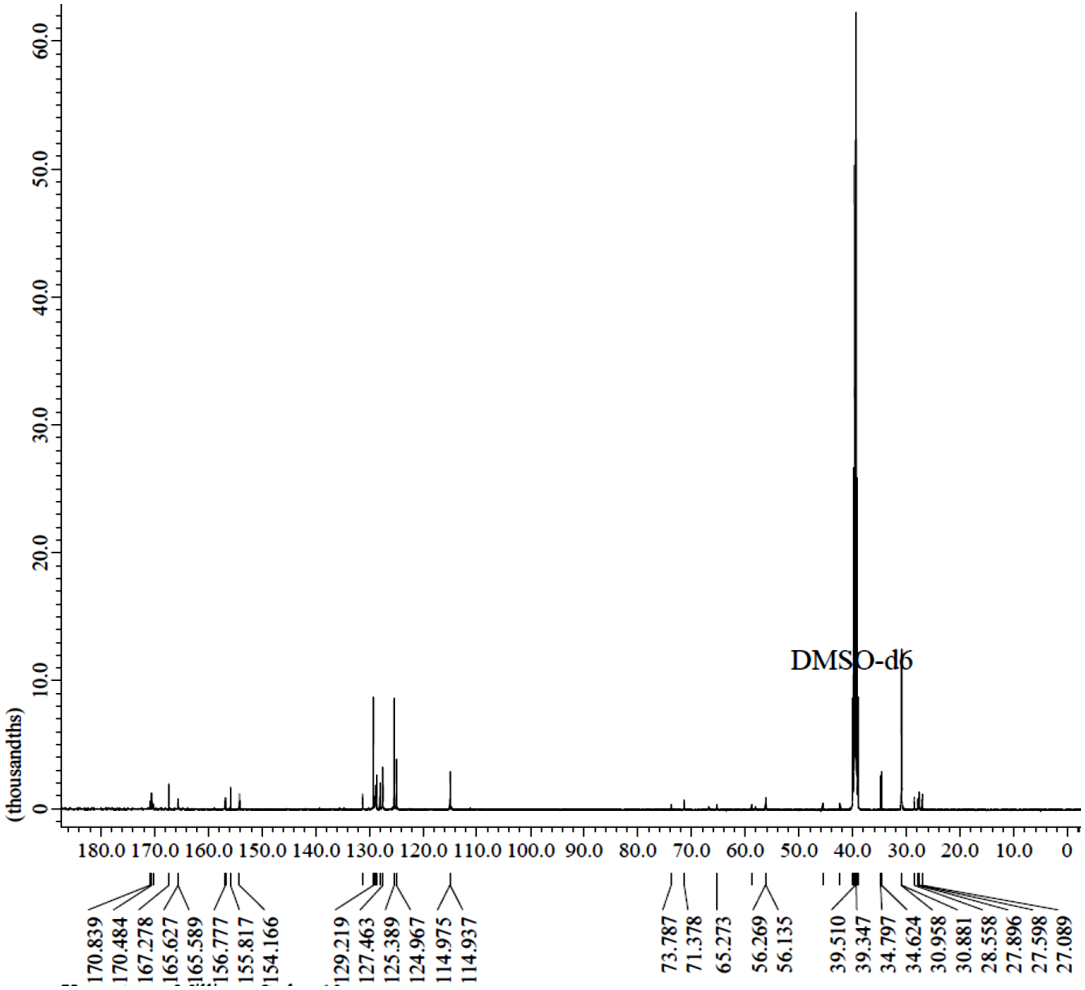


Figure 2. ^{13}C NMR spectrum of 4-*tert*-butylbenzoyl amoxicillin compound

METHODS

Research Materials

The equipment used in this research is the ^{1}H NMR and ^{13}C NMR spectrometers (JNM-ECZ500R/S1), Petri dish, calipers, autoclaves (Westlab), incubators, micropipettes, inoculation needles OSE, Bunsen burner, laminar air flow (stainless steel Indiamart), colony counters, and tools laboratory glassware. The materials used in this study were *E. coli* bacteria, *S. aureus* bacteria, *B. subtilis* bacteria, amoxicillin trihydrate (pharmaceutical grade), 4-*tert*-butylbenzoyl amoxicillin synthesized, ethanol p.a. (Merck), re-distilled water, nutrient agar and nutrient broth, and paper discs.

Synthesis of 4-*tert*-butylbenzoyl amoxicillin compound

After 28.8 mmol of amoxicillin was added with 100 mL tetrahydrofuran and 20 mL, the re-distilled mixture was stirred at 0-5 °C, then 2N potassium hydroxide solution was added. Next, the pH was adjusted between 6.8 to 7.2, followed by adding 25 mmol of 4-*tert*-butylbenzoyl chloride in 40 ml of tetrahydrofuran drop by drop. The mixture was left for several hours at room temperature while stirring occasionally. Next, the tetrahydrofuran in the mixture was evaporated and the solids obtained were dissolved in 300 mL re-distilled water. In the next step, the solution was extracted using 250 mL ethyl acetate. The water phase was separated and in the ethyl acetate phase, as much as 250 mL ethyl acetate was added and cooled. The ethyl acetate in the solution then was evaporated. To remove the remaining ethyl acetate, the solid obtained was dissolved in methanol and then evaporated again to dryness (Siddiq *et al*., 2018).

Characterization of ^{1}H NMR and ^{13}C NMR spectrometry of 4-*tert*-butylbenzoyl amoxicillin compound

A number of dissolved synthesized 4-*tert*-butylbenzoyl amoxicillin compounds were analyzed using `^{1}H NMR and ^{1}C NMR spectrometry with DMSO-d6 solvent.

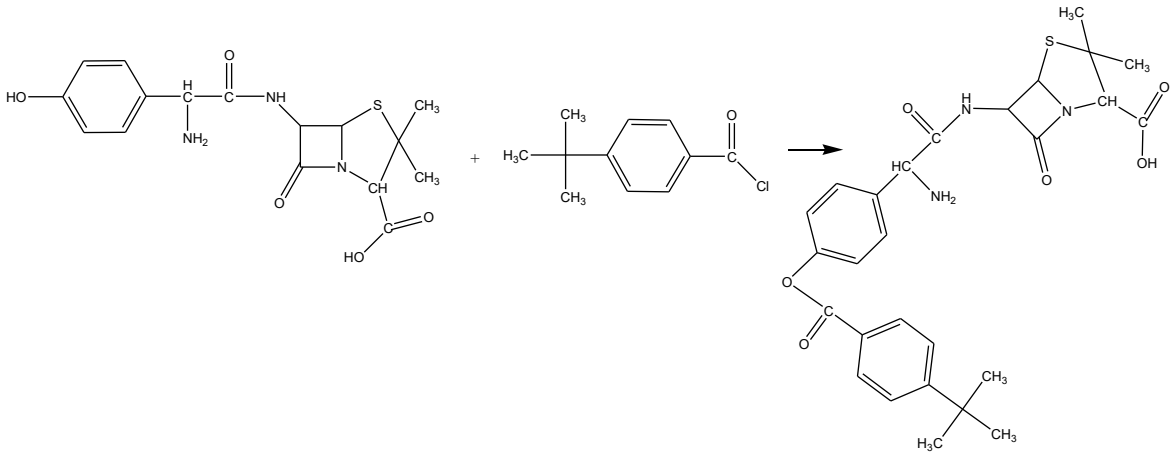


Figure 3. Reaction synthesis 4-*tert*-butylbenzoyl amoxicillin

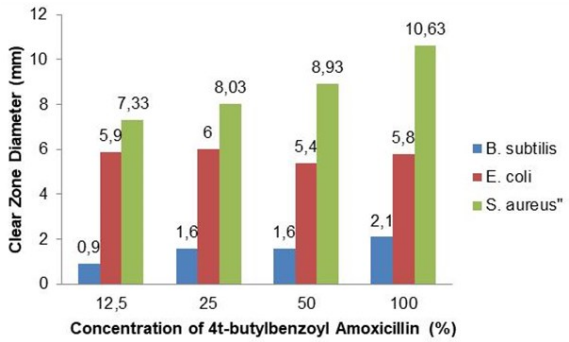


Figure 4. Clear zone diameter of bacteria by 4-tert-butylbenzoyl amoxicillin compound

Antibacterial activity test of 4-*tert*-butylbenzoyl amoxicillin compound

The antibiotic activity test method was the diffusion method using 6 mm diameter paper discs (Kirby-Bauer method). Each compound with a different concentration of 100% (w/v), 50% (w/v), 25% (w/v), 12.5% (w/v) was measured as much as 20 µL and dropped on paper discs until saturated (Ningsih, 2013). The bacterial suspension used was as much as 100 µL to enter into NA media in a sterile Petri dish and then left until it solidified. The saturated disc paper was placed on the surface of the NA media. The media was incubated for 24 hours at 37 °C. Antibacterial activity testing was done in triplicate (Saraswati, 2015).

Antibiotic activity is said to be positive if an inhibitory zone is formed in the form of a clear zone around the disc paper. Inhibition zone measurement is done by reducing the diameter of the resistance with the diameter of the paper disc (Hermawan, 2007).

RESULTS AND DISCUSSION

The results of the ^{1}H NMR spectrum characterization of the 4-*tert*-butylbenzoyl amoxicillin compound are shown in Figure 1 and Table 1. The ^{13}C NMR spectrum of the 4-*tert*-butylbenzoyl amoxicillin compound is shown in Figure 2.

Based on spectrometric data of ^{1}H NMR and ^{13}C NMR, the 4-*tert*-butylbenzoyl amoxicillin compounds were formed by nucleophilic substitution reaction between the hydroxyl group in amoxicillin phenol compound and carbonyl 4-*tert*-butylbenzoyl chloride compound. The reaction that occurs is shown in Figure 3.

The next step was testing the antibacterial activity. Tests were conducted using paper disk diffusion method on *E. coli, S. aureus*, and *B. subtilis* through clear zone observations and the results are shown in Figure 4.

The mechanism of bacterial inhibition is the destruction of the bacterial cell wall. Gram negative bacteria (*E. coli* and *S. aureus*) have a thin layer of peptidoglycan on the cell wall and are surrounded by lipoproteins, lipopolysaccharides, phospholipids, and several proteins. Meanwhile, the gram-positive bacteria (*B. subtilis*) cell walls are composed of tissue with many pores and a thick layer of peptidoglycans and surrounded by a layer of ketoic acid. In Figure 4, gram-negative bacteria are more easily inhibited by the 4-*tert*-butylbenzoyl amoxicillin compound, because it has a thinner layer of peptidoglycan.

In general, the response to inhibition of bacterial growth of 4-*tert*-butylbenzoyl amoxicillin compound is still less effective in providing inhibition of bacterial growth. According to Greenwood (1995) responses to bacterial growth inhibition can be classified as presented in Table 2.

Table 2. Classification of bacterial growth inhibition

Clear zone diameter (mm)	Bacterial growth response
> 20	Strong
16 – 20	Medium
10 – 15	Weak
< 10	Less effective

CONCLUSIONS

The 4-*tert*-butylbenzoyl amoxicillin compound has a specific spectrum for the ^{1}H NMR and ^{13}C NMR test results, so that the mechanism of nucleophilic substitution reaction between the hydroxyl group in the amoxicillin phenol compound and the carbonyl compound 4-*tert*-butylbenzoyl chloride can be explained.

The 4-*tert*-butylbenzoyl amoxicillin compound forms a clear zone against *E. coli, S. aureus*, and *B. subtilis* bacteria and belongs to the less effective category based on the response to bacterial growth inhibition.

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