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Preparation, Characterization and Antimicrobial Activity of Some Amoxicillin Derivatives

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Abstract: According to the World Health Organization, amoxicillin is the most widely consumed antibiotic in the world. In this work new derivatives of amoxicillin were prepared by making some modifications of amoxicillin molecule, based on its sensitivity to penicillinase and *β-lactamase* enzymes secreted by some types of bacteria, the acetyl group was fixed on the amoxicillin molecule as a first step, and then a reaction was performed between the resulting amoxicillin derivative with thiosemicarbazide hydrochloride. The prepared compounds have been investigated on the bases of UV-Vis and IR spectral studies.17 An in vitro antimicrobial investigation was also carried out against four bacteria; *Bacillus subtitles*, *Staphylococcus aureus* (Gram-positive), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative) and one Fungi; *Candida albicans*, to assess their antimicrobial activity by disc diffusion technique. The prepared compounds showed a vital effect against tested bacteria and Fungi.

Keywords: Antibiotics, Amoxicillin, *β-Lactamase*, Thiosemicarbazone, Antimicrobial activities.

I. Introduction

Infectious diseases have been a major health problem for decades, especially in the developing and under developed countries. Moreover, the tendency of microorganisms to develop resistance to antibiotics further necessitates the urge to develop newer drugs. Schiff bases are an important class of organic compounds, synthesized from the condensation of primary amines with carbonyl groups [1]. They were first reported by Hugo Schiff in 1864 [2]. Structurally a Schiff base is a nitrogen analogue of a carbonyl compound in which the carbonyl group is replaced by an imine or azomethine group [3]. The imine group in these compounds is proved to be responsible for various biological activities, antibacterial and antifungal activities being prominent [4]. The general formula of a Schiff base is RHC=N-R1, where R and R1 are alkyl, aryl, cycloalkyl or heterocyclic groups [1]. Amoxicillin, a β-lactam antibiotic belonging to the class of penicillins, is an acid stable semi-synthetic drug shown to be effective against a wide range of infections caused by numerous Gram-positive and Gram-negative strains of bacteria both in humans and animals [5-6]. Originally introduced in the early 1970s for oral use in the U.K, it has gradually established itself as an effective remedy to a wide range of infectious diseases [7]. Amoxicillin monograph is available in the United States, British and Indian Pharmacopeias [8]. It is also on the World Health Organization's list of Essential Drugs [9].

In view of these data, this study focused on the synthesis and biological activity of a new amoxicillin derivatives produced by making some modifications to the amoxicillin molecule, based on its sensitivity to penicillinase and *β-lactamase* enzymes secreted by types of bacteria, which break the *β-lactam* ring that represents the part effective in an antibiotic molecule, and to solve this problem in a first step, the acetyl group is fixed in the amoxicillin molecule by performing the electrophilic substitution reaction (Acylation) with anhydrous acetic acid compound. In a second step, a reaction is made between the resulting amoxicillin

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derivative with thiosemicarbazide hydrochloride to fix a large group on the amoxicillin molecule that may protects the antibiotic from bacterial enzymatic attack during cell intrusion.

II. Materials and Methods

2.1. Chemicals

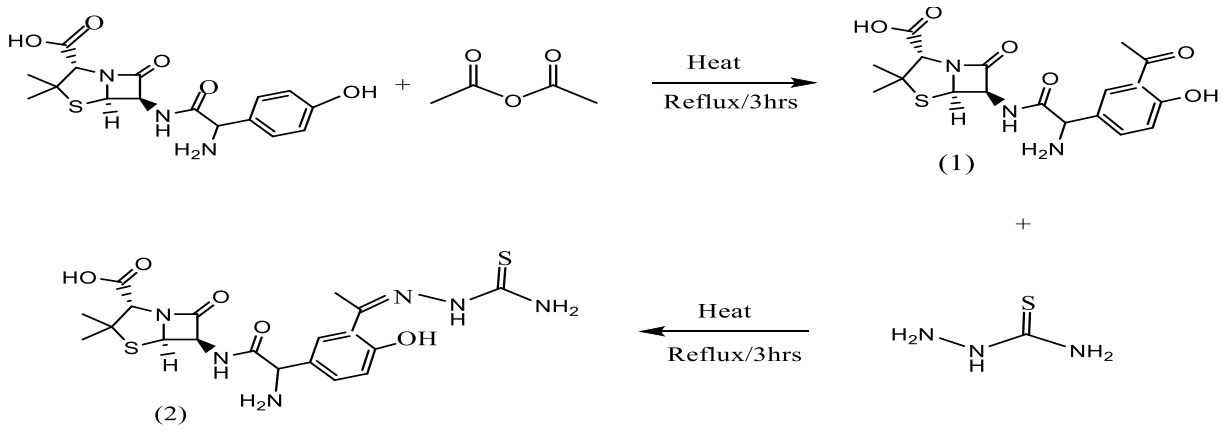
Amoxicillin trihydrate, thiosemicarbazide hydrochloride, anhydrous acetic acid, sodium acetate, hydrochloric acid, ethanol and distilled water.

2.2. Synthesis of amoxicillin derivative (1)

150ml of distilled water was mixed with 5ml of concentrated hydrochloric acid, (6.96g, 16.63mmol) of amoxicillin was added with stirring at 50℃, then (2.7g, 2.5ml) of anhydrous acetic acid was added and waited until the smell disappeared, and then 3g of sodium acetate dissolved in 50ml of distilled water was quickly added, the mixture was cooled in an ice bath, yellow crystals of(2S, 5R, 6R)-6-[(3-acetyl phenyl-4-hydroxy) acetamido]-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo [3.2.0]heptane-2-carboxylic acid were formed, filtered and washed with cold distilled water and dried [10].

2.3. Synthesis of amoxicillin derivative (2)

Synthesis of (2S, 5R, 6R)-6-[(3-acetyl phenyl-4-hydroxy) acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid thiosemicarbazone was based on addition of ethanolic solution (20ml) of amoxicillin derivative (1). (0.0005mol, 0.2312g) to ethanolic solution (20ml) of thiosemicarbazide hydrochloride (0.0005mol, 0.04557g) and the mixture was refluxed at 60℃ for 3hrs. And then the solution was allowed to cool at room temperature and left for slow solvent evaporation. After several days Pale yellow crystals were obtained. The crystals were separated, washed with cold ethanol, and dried.



Scheme (1): Synthesis of amoxicillin derivatives

2.4. IR spectral analysis

The appropriate weight of the amoxicillin and amoxicillin derivatives were taken and grind with potassium bromide to form KBr disk for infrared (IR) spectrum record within the range 400-4000cm^{-1}.

2.5. UV-Vis spectral analysis

A solution with a concentration (1×10^{-3}mol.L^{-}) of amoxicillin and amoxicillin derivatives. The absorbance spectra were recorded in visible and ultraviolet region with 200-800nm by using ethanol as a reference solution.

2.6. Antimicrobial activity

2.6.1. Antibacterial screening

Paper disc diffusion method was used to screen the antibacterial activity of the prepared compounds and performed by using Mueller Hinton agar (MHA). The antibacterial screening was carried out according to the National Committee for Clinical Laboratory Standards Guidelines. Bacterial suspension was diluted with sterile physiological solution to 108 cfu/mL (Turbidity=Mc Farland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on the surface of MHA and the inoculum was allowed to dry for 5 minutes.

Sterilized filter paper discs (Whatman No. 1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20µL of a solution of each sample. The inoculated plates were incubated at 37°C for 24hrs in the inverted position. The diameters (mm) of the inhibition zones were measured [11].

2.6.2. Antifungal screening

Preliminary antifungal screenings of the prepared compounds at different concentrations were performed. Potato dextrose agar medium was prepared by using potato, dextrose, agar-agar and distilled water. Appropriate amount of the compounds in DMSO was added to potato dextrose agar medium in order to get a concentration of 5% µg/mL of compound in the medium. The medium was poured into a set of two Petri plates under aseptic conditions in a laminar flow hood. When the medium in the plates was solidified, a mycelial disc of 0.5 cm in diameter was cut from the periphery of the seven days old culture and it was aseptically inoculated upside down in the center of the Petri plates. These treated Petri plates were incubated at 26±1 °C until fungal growth in the control Petri plates was almost complete. The mycelial growth of fungi (mm) in each Petri plate was measured [12].

III. Results and discussion

3.1. IR data of amoxicillin and amoxicillin derivatives

The IR spectrum of the amoxicillin showed absorption peak at 1776 cm^{-1} due to the expansion of the association C=O and note that the decrease of this absorption in amoxicillin derivatives, indicating increase the size of the structure, and the compounds showed absorption peaks at 2682-3460 cm^{-1} and 2852-2970 cm^{-1} are due to the NH and NH_2 groups respectively, and the appearance of absorption peaks at 723-800 cm^{-1} are due to C-S, absorption peaks at 2918-3527 cm^{-1} are due to O-H group[13], and the ligand showed absorption peaks at 1631cm^{-1}, 1523 cm^{-1} and 1467 cm^{-1} are due to the stretching of conjugate C=N, C=S and absorption N-C-N groups respectively [14].

Table (1): IR spectrum of the amoxicillin and amoxicillin derivatives

No	Compounds	v(C=O)	v(NH_2)	v(NH)	v(C-S)	v(-OH)	v(C=N)	v(C=S)	v(N-C-N)
1	Amoxicillin	1776	2970	3460	800	3527	-	-	-
2	Derivative (1)	1703	2852	2682	723	2918	-	-	-
3	Derivative (2)	1701	2852	3174	800	2920	1631	1523	1467

Table (2): U.V spectrum of the amoxicillin and amoxicillin derivatives

No	Compounds	Absorption bands n-π* in (nm)	Absorption bands π-π* in (nm)
1	Amoxicillin	670, 620,479,310	274,238
2	Derivative (1)	638,622,589,357	273,233
3	Derivative (2)	640,620,610,590,483	342

3.2. Antimicrobial activity

The synthesized compounds were screened in vitro for their antimicrobial activity against four pathogenic bacteria; *Bacillus subtitles*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and one fungus; *Candida albicans* at a concentration of 5 µg/mL with DMSO as the solvent. The results showed that the tested compounds possess moderate antimicrobial activity against most of the tested organisms, as shown in Table (3).

Table (3): Antimicrobial activity of amoxicillin and amoxicillin derivative and its ligand

No	Compounds	**Conc µg/Ml**	**E.c**	**Ps.a**	**S.a**	**B.s**	**C.a**
1	Amoxicillin	5	15	14	11	16	19
2	Derivative (1)	5	14	14	10	09	10
3	Derivative (2)	5	21	23	20	20	30

Gram positive bacteria; B.s = *Bacillus subtitles*, S.a = *Staphylococcus aureus*, Gram negative bacteria; E.c = *Escherichia coli*, Ps.a = *Pseudomonas aeruginosa and* Fungi; C.a = *Candida albcans*.

The results were expressed in terms of the diameter of the inhibition zone: <9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.

IV. Conclusion

In this study we have synthesized two new biologically active amoxicillin derivatives. The synthesized compounds were studied on the basis of physical and spectral data, and they have antibacterial and antifungal activities and inhibit the action of microbes and at the same time prevent bacterial enzymes from inhibiting the action of the compounds.

V. References

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