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Synthesis, characterization and comparative study the antibacterial activities of some imine-amoxicillin derivatives

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ABSTRACT

In this study, we report the synthesis of some new Schiff base compounds (1-5) from the reaction of amoxicillin with some aromatic aldehydes in classical Schiff base method. These derivatives were characterized by melting point, elemental analysis, FT-IR and ^{1}H NMR data. All the synthesized compounds were evaluated *in vitro* for their antibacterial activities against two Gram positive (*Staphylococcus aureus, Streptococcus faecalis*) and two Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) microorganisms in different concentrations (10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} M) by agar diffusion disk method. The results showed that some of these derivatives have good antibacterial activities compared to biological activity of parent drug.

1. Introduction

Amoxicillin (penicillin A), Figure 1, a very popular, safe antibiotic and is usually the drug of choice within the class because it is better absorbed compared to other beta-lactam antibiotics [1]. It is a moderate-spectrum, bacteriolytic, β-lactam antibiotic used to treat many different types of infections caused by susceptible microorganisms. it is active against a wide range of Gram-positive, and a limited range of Gram-negative organisms [2]. It is also found that amoxicillin and its derivatives are very importance in many research studies, such as pharmaceutical and biological studies [3-6].

Compounds containing an azomethine group (imine) are a class of important compounds in medicinal and pharmaceutical field. The biological applications of these compounds have attracted remarkable attention [7]. Some Schiff-bases were exhibits antibiotic, antiviral and antitumor agents because of their specific structure [8]. The wide use of antibiotics resulted in the serious medical problem of drugs resistance and public health concern. The synthesis of new derivatives of antibiotics has become an important task to cope with drug resistance problems [9,10].

Due to the activities associated with amoxicillin and imines, an attempt was made to generate new derivatives containing imine and amoxicillin in the same molecules (**1-5**). All the synthesized compounds were further characterized and evaluated for antibacterial activities.

There are many interesting studies on the Schiff bases compounds and their complexes derived from antibiotics. Naz and Iqbal [11] found that the Schiff base complexes derived from amoxicillin having good antibacterial activity in good range when comparison to control (Amoxicillin). Also Joshi *et. al.* [12] synthesized two ligands derived from amoxicillin and their complexes with many metals that it used in biological activates. He found that the Schiff base ligands were found to be biologically active and their metal complexes display enhanced antimicrobial activity.

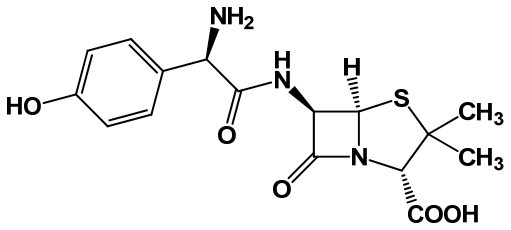

Figure 1. Amoxicillin structure.

2. Experimental

2.1. Materials and physical measurements

All reactants and solvents used in this study were reagents grade and they are available from Sigma-Aldrich and Fluka companies. Amoxicillin trihydrate was supplied by Arab Company for Antibiotics Industries (ACAI) in Iraq.

Melting points were determined on Electro-thermal capillary apparatus and are uncorrected. Elemental analysis (C, H, N) were carried out using a Euro-Vector model EA 3000 A instrument. The FT-IR spectra were obtained using SHIMADZU model FT-IR-8400S. ^{1}H NMR spectra were obtained on BRUKER model Ultra shield 300 MHz spectrophotometer in DMSO-d_6 solution with the TMS as internal standard.

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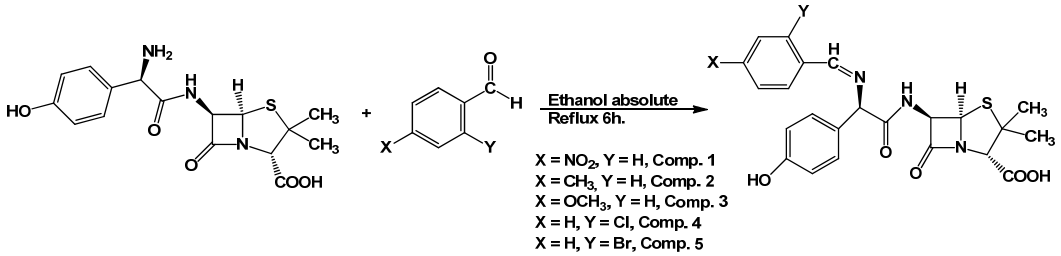
Table 1. Physical properties, elemental analysis and FT-IR data of compounds (**1**-**5**).

Compound	M.p., °C	Yield, %	FT-IR, cm^{-1}	Elemental analysis, calculated (found), %		
			CH=N	C	H	N
1	179-182	77	1664	55.41 (55.23)	4.45 (4.21)	11.24 (11.16)
2	209-211 (Dec.)	62	1662	61.65 (61.70)	5.39 (5.51)	8.99 (9.04)
3	218-220	67	1664	59.61 (59.66)	5.21 (5.12)	8.69 (8.61)
4	215-216	71	1663	56.61 (56.50)	4.54 (4.60)	8.61 (8.55)
5	188-190	83	1666	51.89 (52.02)	4.16 (4.27)	7.89 (7.95)



Scheme 1

2.1.1. General method for the preparation of (5R,6R)-6-{[(E)-substitutedbenzilidene]amino}-2-(4-hydroxyphenyl) acetyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid (1-5)

The Schiff bases derived from substituted benzaldehydes and amoxicillin trihydrate were prepared using a modified method similar to one given by Joshi *et al.* and Al-Garawi *et al.* [12,13]. A solution of substituted benzaldehydes (1.1 mmol) in ethanol (10 mL) and amoxicillin trihydrate (0.419 g, 1.0 mmol) were mixed. The obtained solution was then magnetically stirred and refluxed for 6 h at boiling temperature. The obtained brownish red solution was poured in cold water (100 mL). The solid was filtered and washed several times with water then dried in air for 2 h.

2.1.2. General method for determination the antibacterial activity of Schiff base compounds (1-5)

The prepared compounds (**1**-**5**) were screened for antibacterial activities using cup-plate agar diffusion method by measuring the inhibition zone in mm [14,15]. Amoxicillin in different concentrations (10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} M) was used as a standard drug for comparative antibacterial activity of the prepared compounds. The compounds were screened for antibacterial activity against two Gram positive (*Staphylococcus aureus, Streptococcus faecalis*) and two Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) microorganisms that have been isolated from infected wounds, nose swab, urinary tract infection, surgical theaters, respectively, in Muller Hinton agar. These sterilized agar media were poured into Petri-dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 12 mm diameter (pre-sterilized) was used to bore cavities. All the synthesized compounds in different concentrations (10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} M) were placed serially in cavities with the help of micropipette and allowed to diffuse for one hr. DMSO was used as a solvent for all compounds and sterile distilled water was used as a solvent for pure amoxicillin. These plates were incubated at 37 °C for 48 hr. The zone of inhibition observed around the cups after respective incubation was measured in mm.

3. Results and discussions

3.1. Synthesis

Scheme 1 outline the synthetic sequences employed in our laboratories for preparation of Schiff base compounds (**1**-**5**). The Schiff base derivatives of amoxicillin were prepared in good yield by the reaction of the corresponding benzaldehydes with amoxicillin trihydrate in absolute ethanol as a solvent. The structural assignment of amoxicillin trihydrate was based on melting point (182-184 °C decomposed) and their spectral FT-IR spectroscopy. The FT-IR spectrum of this antibiotic exhibited significant two bands at 3332 and 3163 cm^{-1} which could be attributed to asymmetric and symmetric stretching vibrations of NH_2 group. Besides this, bands about 1776 and 1687 cm^{-1} are due to C=O stretching for lactam and carboxyl groups respectively. It also showed a broad band between 3041-2621 cm^{-1} due to stretching O-H of carboxyl group (υ COOH). The bands that appeared at 3527 and 3462 cm^{-1} were assigned to the amide (N-H) and hydroxyl group (O-H) respectively.

The FT-IR spectra of compounds (**1**-**5**) indicated the presence of CH=N function (1662-1666 cm^{-1}) and disappearances two bands at (3332 cm^{-1}) and (3163 cm^{-1}) which could be attributed to asymmetric and symmetric stretching vibration of NH_2 group of amoxicillin. Reviews on ^1H NMR of Amoxicillin.$3H_2O$ in DMSO-d_6 solvent is available in references [16,17]. The amine and carboxyl protons did not observed in the spectrum because the extent of intermolecular interactions with DMSO-d_6 [18,19]. The ^1H NMR spectra of these compounds **1**-**5** showed a singlet at δ 10.03, 9.98, 9.93, 9.87 and 10.29 ppm due to the imines protons, respectively. The physical properties and FT-IR data for Schiff bases derivatives are listed in Table 1 and the ^1H NMR data are listed in Table 2.

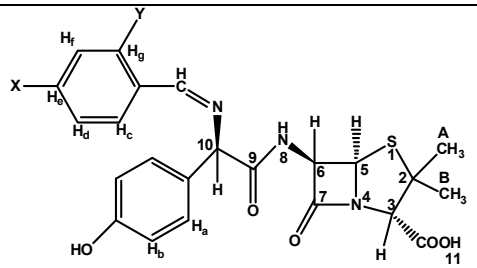
3.2. Antibacterial studies

All the synthesized compounds (**1**-**5**) with parent drug (Amoxicillin tri hydrate) have been screened in vitro for antibacterial activities.

When we examine the data of (inhibition zone) of all compounds (**1**-**5**) against *Staphylococcus aureus* in Figure 2, we observe some important results: that the compounds **3**, **4** and **5** showed biological activities more than the parent drug and that these activities increases with increasing the concentration of these compounds while compounds **1** and **2** showed the opposite. Also, the compound **3** has a biological activity higher than the compounds **4** and **5**.

We note from Figure 3, the activity against *Streptococcus faecalis*, that all the prepared compounds, except compound **1**, have higher biological activities than the pure amoxicillin and this efficiency increases with increasing the concentration of compounds, also the compound **5** has the highest activity against this type of bacteria compared to others.

Table 2. [1]H NMR data of compounds (**1**-**5**) [a].



Assignment	Amoxicillin.3H$_2$O [b]	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
A-CH$_3$	1.38 (s)	1.31 (s)	1.21 (s)	1.32 (s)	1.34 (s)	1.39 (s)
B-CH$_3$	1.49 (s)	1.56 (s)	1.48 (s)	1.50 (s)	1.48 (s)	1.54 (s)
H-3	3.98 (s)	3.99 (s)	4.39 (s)	3.86 (s)	3.97 (s)	3.99 (s)
H-10	4.87 (s)	4.76 (s)	4.82 (s)	4.79 (s)	4.81 (s)	4.83 (s)
H-5	5.32 (d)	5.29 (d)	5.39 (d)	5.44 (d)	5.33 (s)	5.37 (s)
H-6	5.50 (s, bd)	5.68 (s, bd)	5.51 (s, bd)	5.66 (s, bd)	5.69 (s, bd)	5.61 (s, bd)
H$_a$	6.77 (d)	6.74 (d)	6.69 (d)	6.67 (d)	6.68 (d)	6.70 (d)
H$_b$	7.30 (d)	7.33 (d)	7.21 (d)	7.25 (d)	7.23 (d)	7.20 (d)
NH	8.68 (d)	8.61 (d)	8.59 (d)	8.56 (d)	8.63 (d)	8.61 (d)
OH	9.05 (d)	9.16 (d)	9.11 (d)	9.14 (d)	9.09 (d)	9.12 (d)
NH$_2$	Not observed	-	-	-	-	-
CH=N	-	10.03 (s)	10.01 (s)	9.98 (s)	10.02 (s)	10.12 (s)
COOH	Not observed	11.07 (s)	11.09 (s)	11.10 (s)	11.02 (s)	11.04 (s)
H$_c$	-	7.81 (d)	7.79 (d)	7.86 (s)	7.61 (d)	7.66 (d)
H$_d$	-	8.32 (d)	8.37 (d)	8.40 (d)	8.18 (t)	8.16 (t)
H$_e$	-	-	-	-	8.27 (t)	8.22 (t)
H$_f$	-	8.32 (d)	8.37 (d)	8.40 (d)	8.36 (d)	8.41 (d)
H$_g$	-	7.81 (d)	7.79 (d)	7.86 (d)	-	-
X	-	-	2.26 (s)	4.04 (s)	-	-
Y	-	-	-	-	-	-

[a] Abbreviations: s=singlet; d=doublet; t=triplet; bd=broad.
[b] The data was taken from the references [16,17].

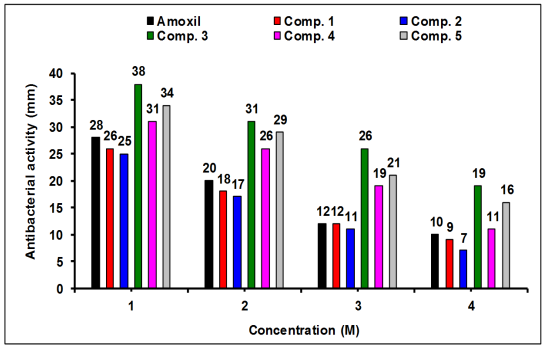


Figure 2. The activity of compounds (**1**-**5**) against *Staphylococcus aureus* in different concentrations (1 = 10^{-1}, 2 = 10^{-3}, 3 = 10^{-5}, and 4 = 10^{-7} M).

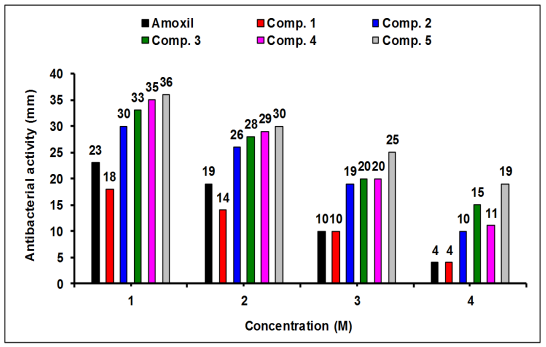


Figure 3. The activity of compounds (**1**-**5**) against *Enterococcus faecalis* in different concentrations (1 = 10^{-1}, 2 = 10^{-3}, 3 = 10^{-5}, and 4 = 10^{-7} M).

When comparing the biological activities of two types of Gram possitive (*Staphylococcus aureus* and *Streptococcus faecalis*) for all compounds we find that compound **3** retains its high effectiveness among all the derivatives and show potent activity against *Staphylococcus aureus*.

On the other hand, when we show the results of activity against *Escherichia coli*, Figure 4, we observed some important notes: the first that all compounds (**1**-**5**) in all concentrations have good activity against this bacteria and this inhibition increases with increasing the concentration of compounds. The second is the compound **5** has good activity compared to others.

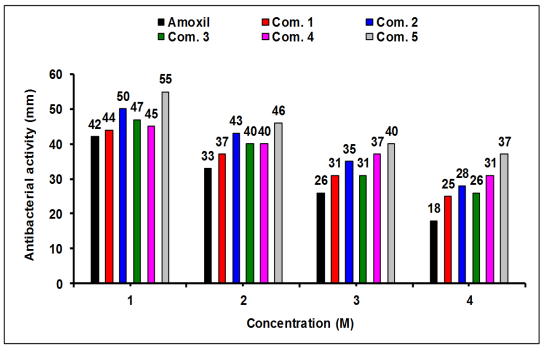


Figure 4. The activity of compounds (**1**-**5**) against *Escherichia coli* in different concentrations (1 = 10^{-1}, 2 = 10^{-3}, 3 =10^{-5} and 4 = 10^{-7} M).

When we assess the results of the biological activity of the compounds (**1**-**5**) against *Pseudomonas aeruginosa*, Figure 5, we find that only two compounds (**2** and **3**) have shown the activity higher than amoxicillin, while compounds **4** and **5** showed less activity than pure amoxicillin note that with the

activity of compound **1** towards these bacteria almost similar to the activity of parent drug.

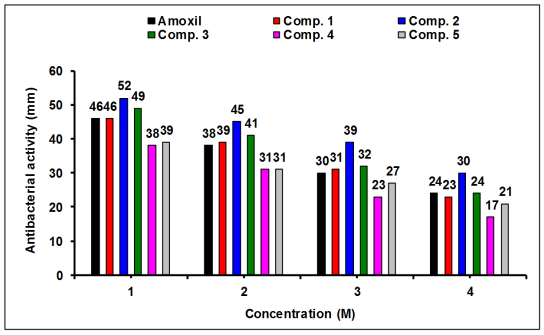


Figure 5. The activity of compounds (**1**-**5**) against *Pseudomonas aeruginosa* in different concentrations (1 = 10^{-1}, 2 = 10^{-3}, 3 = 10^{-5}, and 4 = 10^{-7} M).

4. Conclusions

Schiff base compounds derived from amoxicillin trihydrate were prepared and structurally characterized using spectroscopic techniques. The synthetic route started from reaction between amoxicyllin and appropriate substituted benzaldehydes (4-nitro benzaldehyde, 4-methyl benzaldehyde, 4-methoxy benzaldehyde, 2-chloro benzaldehyde and 2-bromo benzaldehyde) in ethanol. The Schiff base compounds containing amoxicillin moiety have been evaluated *in vitro* for their antimicrobial activities against two types of bacteria: Gram positive (*Staphylococcus aureus, Streptococcus faecalis*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) microorganisms in different concentrations (10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} M). The results showed that some of these derivatives have good antibacterial activity compared to the parent drug.

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