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RESEARCH ARTICLE

Pharmacological Study of Schiff Base Derived from Amoxicillin Drug and Vanillin

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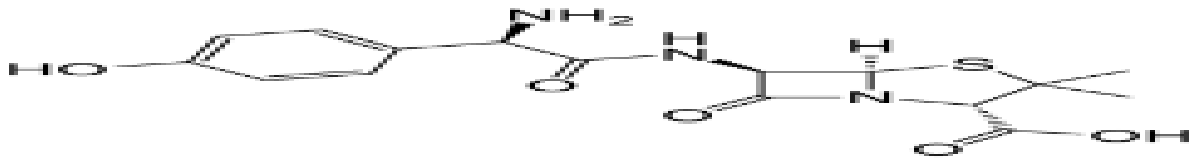
Abstract

Objective: Our present investigation is based on development and synthesis for new derivatives of amoxicillin as the wide use of this antibiotic led to serious medical problem of drugs resistance. Methods: Condensation of amoxicillin trihydrate with 4-Hydroxy-3-methoxy benzaldehyde yielded a novel Schiff base derivative of amoxicillin in good yield. Characterization of synthesized compound detected by IR and ^{1}HNMR spectroscopy. The antibacterial activity of new compound was screened against *the* selected bacteria species *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus, and Klebsella pneumonia*. The LD_{50} value was determinate by using up and down method. Results: Our obtained results for this antibiotic modulation method are within high acceptance criteria *in vivo* study determination of the toxic effect (LD_{50}) of synthesized compound which was 477.2 mg / kg of body weight give a moderate value. Furthermore, in vitro study included antibacterial activity against some bacteria species of new compound was higher than the respective standard drug at tested doses level. Conclusion: Modulating amoxicillin drug can be used in the management of bacterial resistant disorders.

Key words: *Amoxicillin, Micro-organism species, Antibacterial activity, Disorders. Schiff-base.*

Introduction

Amoxicillin is semi synthetic drug with in a class of antibiotics known as Penicilin (β-lactams-antibiotics). It is effective against a broad spectrum of micro-organism (gram positive and negative bacteria) which is responsible of a wide range of infections in both human and animals [1]. Amoxicillin is closely related to ampicillin with the same spectrum of activity and potency. It is more effective against gram positive than gram negative micro-organisms. Susceptibility of amoxicillin to degradation by ß-lactamase-producing bacteria, could be overcome by adding ß-lactamase inhibitor such as clavulanic acid but this combination seems to be associated with a higher risk (Stevens-Johnson syndrome, purpura and hepatitis) [2]. Although there are currently new developmental pathways, but most of that are at a pre-clinical stage. A further objective at that time is to enhance amoxicillin formulations that allowed amoxicillin (alone and in combination) to play an important role in the treatment of various infectious disease. The β-lactams (like amoxicillin) antibiotics considered as vital antibiotics due to presence of tetracycleβ-lactam atoms [3]. Chemical structure of amoxicillin fig.1 have 4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, 6-[[amino (4-hydroxy phenyl) acetyl] amino]-3, 3-dimethyl-7-oxo, trihydrate, ($C_{16}H_{19}O_5S$). $3H_2O$ [4].



.$3H_2O$

Fig. 1: Structural formula of amoxicillin trihydrate (AMX)

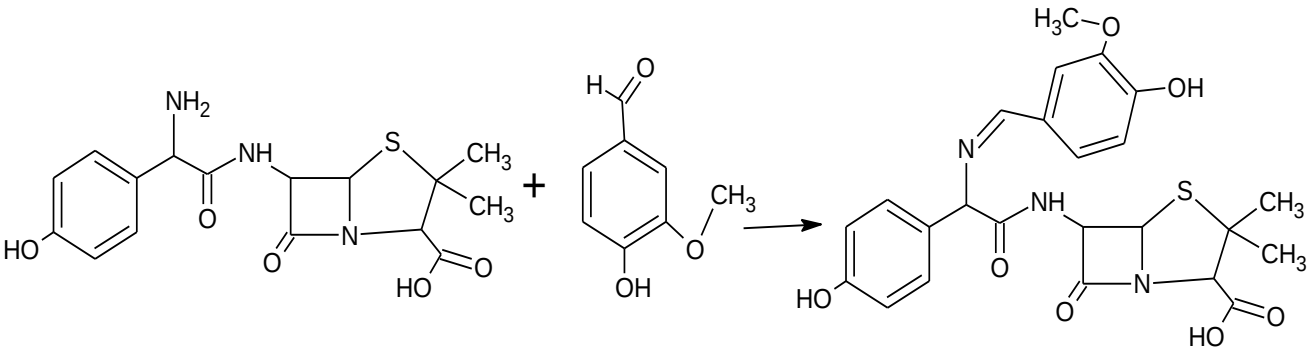
Amoxicillin trihydrate (AMX) have bio-functional activity related to lactam ring that cause bacterial growth inhibition via proteolysis mechanism [5]. AMX structure (including of amide and amino group) suggest that is the better starting compound for the synthesis of new Schiff base. An imine group containing compound is a class of important compounds in medicinal and pharmaceutical research [6].

An attempt to produce new derivatives of improved activity and possible stability against ß-lactam is realized in 2016 by Mohammed M.J [7] by synthesis of six different Schiff bases from ampicillin and amoxicillin. In our study, the antibacterial activity of new amoxicillin derivative was evaluated as well as its acute toxicity.

Material and Methods

Synthesis and Characterization of Novel Schiff Base

Preparation of Schiff base was obtained by mixing a solution of AMX (about 2.247 gm, 5 mmole) in methanol (10 ml) and 4-Hydroxy-3-methoxybenzaldehyde (about 0.76 gm, 5mmole) in equal amount of ethanol, two drops of glacial acetic acid added to the mixture and refluxed for (5 h) with stirring , allowed to cool and dried at room temperature. The product was re-crystallized from ethanol Pale-yellow product Yield; 79%, M.P. = 148. FT-IR (KBr., cm-1), 3362-3215 (OH, NH); 3070 (C-H, aromatic); 2971,2930 (C-H, aliphatic); 1835 (C=O Carboxylic); 1667 (C=O, β-Lactam);1630(C=N); 1611 (C=C); 1592, 1387 (COOH). 1HNMR (DMSO -d6);δ9 .76(s,1 H,COOH) ;8.51 (s,1H,CH=N);8.20(s,1H,NHamide); 7.38-6.71(m,7H,Ar-H); 5.51, 5.63 (s, 2H, Ar-OH); 4.83 (s, 1H, CH-COOH); 4.83, 3.83(m,2H-CH,β-lactam); 3.51(1H,CH-N); 1.54 (s,3H-OCH3); 1.28, 1.17(s,6H, 2CH3). Anal. For C24H25N3O7S (499.55): C, 57.65, H, 5.00, N, 8.40, Found: C, 57.92, H, 5.39, N, 8.71. Scheme 1.



Scheme 1: Synthesis of amoxicillin Schiff base

6-({[(*Z*)-ethylideneamino] (4-hydroxyphenyl) acetyl} amino)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid.

Chemical Analysis

FTIR, ^{1}H-NMR, and elemental microanalysis (CHN) analysis used to confirm the chemical structures of the newly synthesized compound. The characteristic Infrared spectra (IR) band for this compound displayed at range of 4000-400 cm^{-1} (KBr discs) by using FT-IR spectrophotometer Shimadzu model IR. Affinity-1 ^{1}H, ^{13}C NMR spectra were measured to detect methyl groups (using Brucker at 300 MHz, with TMS). Microanalysis for (C=N-CH) were carried out by a Perkin-Elmer 240B elemental analyzer. Determinations of melting points of newly product were done using Philip Harris melting point apparatus.

Antibacterial Activity

The antibacterial activity of synthesized products was assigned against four bacteria species *(Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus cereus*) by using the paper disc-agar diffusion technique [8]. Overnight cultures of selected bacteria were kept at 37ºC for 24 h in the incubator. After that, bacterial suspension was diluted with sterile physiological solution. Petri plates containing 20 ml of Mueller Hinton Agar were then used for all the bacteria tested.

On the other hand three serial dilutions (dimethylsulfoxide DMSO) with concentrations (300,400 and 500µg/ml) of parent and synthesized substances. Filter paper discs 7 mm (Whatman, no. 3) were impregnated with 20 ml of each of the different dilutions.

The discs were allowed to remain at room temperature until complete diluents .Discs with natural and synthesized products placed onto the surface of the agar. After 24h of incubation, confluent bacterial growth was observed. Inhibition zone of the bacterial growth was measured in mm.

Acute Toxicity (LD50)

In vivo study, acute toxicity experiments were performed on old male albino rats weighing between 200-220gm depending on using up-and-down method [9]. Male rats were injected intra peritoneal with different doses of the synthesized compound after conducting series of test levels. With equal spacing between doses; and depending on Dixon method a series of trails were carried out. Testing continued until LD_{50} were determined after reading final result then management of the results as in Table 1, and applying the following equation:

LD_{50} = XF + Kd. (XF; Last dose used in the experiment, d; Difference between doses).

Table 1: Dixon values

	K represented serial tests started with				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544	1.549-	OOOX
XXXX	0.555	0.0897	0.985	1.000	OOOO
	X	XX	XXX	XXXX	
	K represented serial tests started with :-				

Keynotes: (X) response-dead animal. (O) non-response-alive. (K) Factor of change from the table

Results and Discussion

In Vitro Study

Chemistry

The newly Schiff base compound was obtained by reacting amoxicillin trihydrate 4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, 6-[[amino (4-hydroxy phenyl) acetyl]amino]-3,3-dimethyl-7-oxo, trihydrate with vanillin (4- Hydroxy- 4- methoxy benzaldehyde) in methanol with reflux for 5 h in 1:1 mole ratio gave the product having the chemical structure 6-([(*Z*)-ethylideneamino] (4-hydroxyphenyl) acetyl} amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid Scheme.1. Infrared spectrum (IR) of compound displayed characteristic bands at 3362-3215cm^{-1} because of ν (O-H) and ν (N-H) secondary amine stretching vibration and the bands for the ν (N–H) primary amine stretching vibration was disappeared.

The band at 1630cm^{-1} was due to azomethine group ν(-HC=N-) stretching vibrations, while, the band at 1835 cm^{-1} was due to ν(C=O) cm^{-1} stretching vibration of CO_2H group. This Schiff base showed the band at 1667 cm-1 stretching vibration due to ν(C=O) for ß-lactam group overlapping with ν(-HC=N-) stretching vibrations. The ^{1}HNMR spectrum of synthesized compound showed that all the expected protons with proper intensity ratio. The singles at δ = 1.28 and 1.17 ppm were attributed to methyl protons and methoxy protons were appeared at δ =1.54. The aromatic protons appeared in the region δ = 7.38-6.71 ppm. The protons of hydroxyl groups of Ar-OH and -COOH were assigned as two singlet's at δ = 5.51 and 5.63 ppm, respectively. The proton of azomethine (CH=N) resonated as a singlet at δ = 8.51 ppm. Three groups of double peaks given by (CO–CH) and (N–CH) on the β-lactam ring and (NH sec.) amide appeared at δ = 4.81, 3.83 and 8.20 ppm, respectively.

Antibacterial Activity

The antibacterial activity results are shown in Table 2, Synthesized Schiff base was active against all the selected bacteria species with different values according to concentration differentiation. Natural compound inactive against *E. coli* and *staphylococcus aureus* Fig. 1 and 2, respectively.

In comparison with obvious activity in all concentration of new compound, the synthesized compound showed high activity in *Bacillus sp*. at all concentrations. On the other hand, the studied amoxicillin have no activity at 500 µg/ml concentration Fig. 3.

The new compounds Fig.4 have a moderate activity against *Klebsella sp*. While, the amoxicillin derivative showed high degree of activity at all concentration. This study clearly demonstrated that the synthesized Schiff base of amoxicillin and vanillin showed significant antibacterial activity, particularly against *E. coli* and *staphylococcus aureus* compared with the parent drugs that have no activity against them.

Table 2: Antibacterial Activity of Amoxicillin and New compound

Compound	Bacteria sp.	*E. coli*			*Bacillus. aureus*			*Staph. aureus*			*Kleb. pneumonia*		
	Conc.(µg/ml	300	400	500	300	400	500	300	400	500	300	400	500
Amoxicillin		-	-	-	15	31	-	-	-	-	33	29	33
New compound		28	29	31	28	32	25	30	23	26	24	20	26

Keynotes: Bacteria sp. Bacteria species. (Conc) concentration

In Vivo Study

Median Lethal Dose (LD50)

In- vivo study as a part of our experiment conclude evaluated of the 50% of lethal dose (LD50) of the synthesized compound *so* this part applied on laboratory albino rats as model using the method described by Dixon [10]. In the experiment we using 10 animals of white adult rats, and we started using serial graduated doses for injection of studied compound. Administration of series of concentrations (150, 200, 250, 300,…., 2500 mg/kg b.w) dissolved in 0.1 ml DMSO, were applied and chosen with equal spacing (about 50mg) between doses. After 24h of administration mortality was recorded and so, acute toxicity of studied compound was recorded by applying formula and the result of LD50 was as below:

LD50 =X*f*+ K

LD50 = 400 + (1.544) x50

LD50 = 477.2 mg / kg b.w

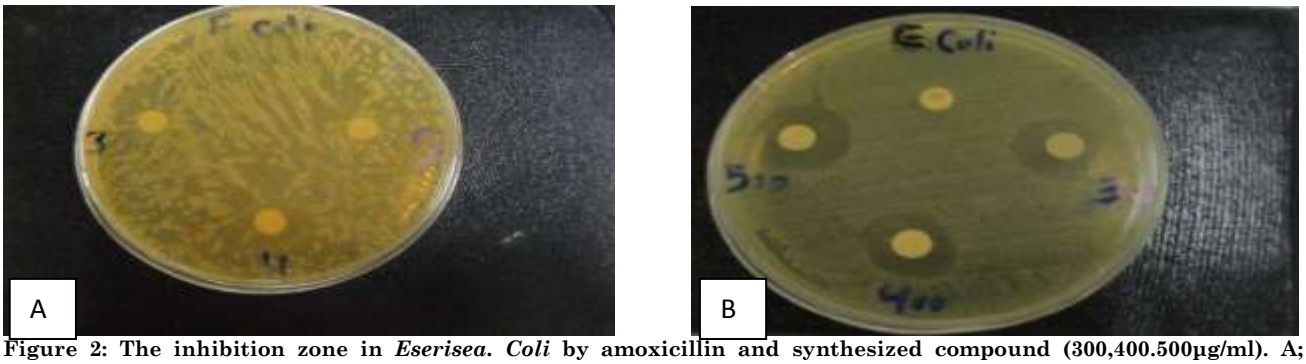


Figure 2: The inhibition zone in *Eserisea. Coli* by amoxicillin and synthesized compound (300,400.500µg/ml). A; Amoxicillin. B; New compound

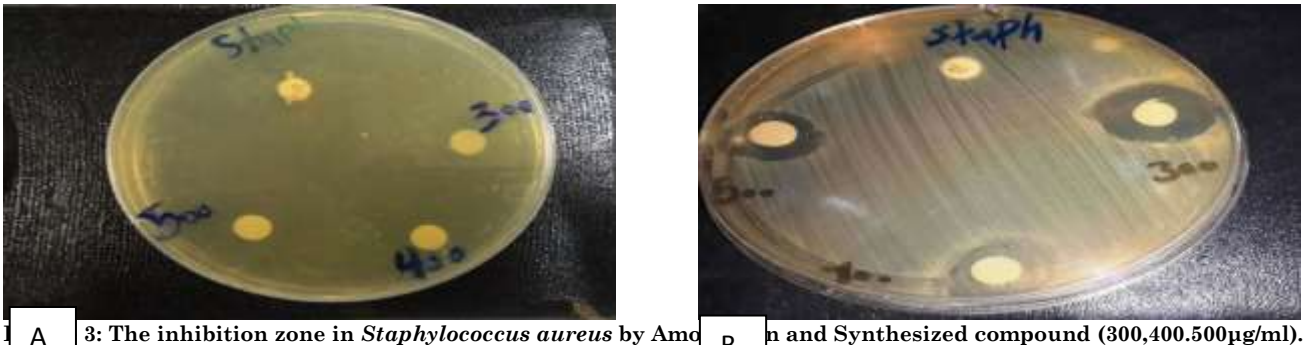


Figure 3: The inhibition zone in *Staphylococcus aureus* by Amoxicillin and Synthesized compound (300,400.500µg/ml). A; Amoxicillin. B; New compound

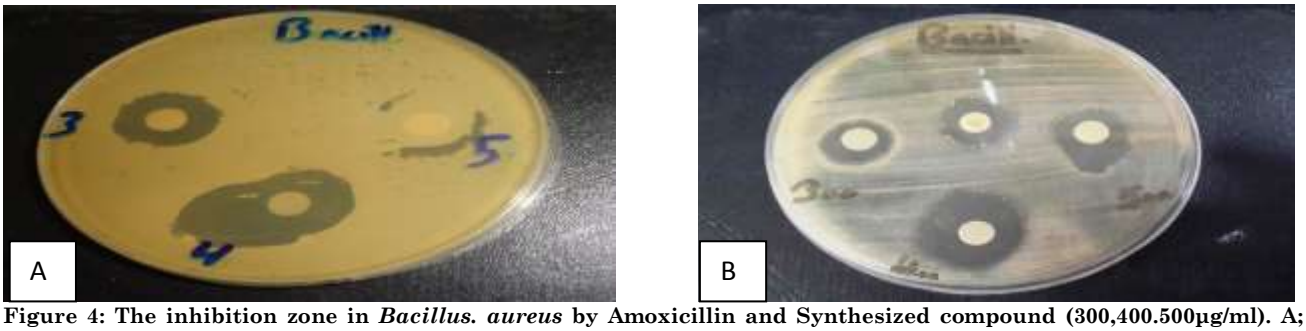


Figure 4: The inhibition zone in *Bacillus. aureus* by Amoxicillin and Synthesized compound (300,400.500µg/ml). A; Amoxicillin. B; New compound

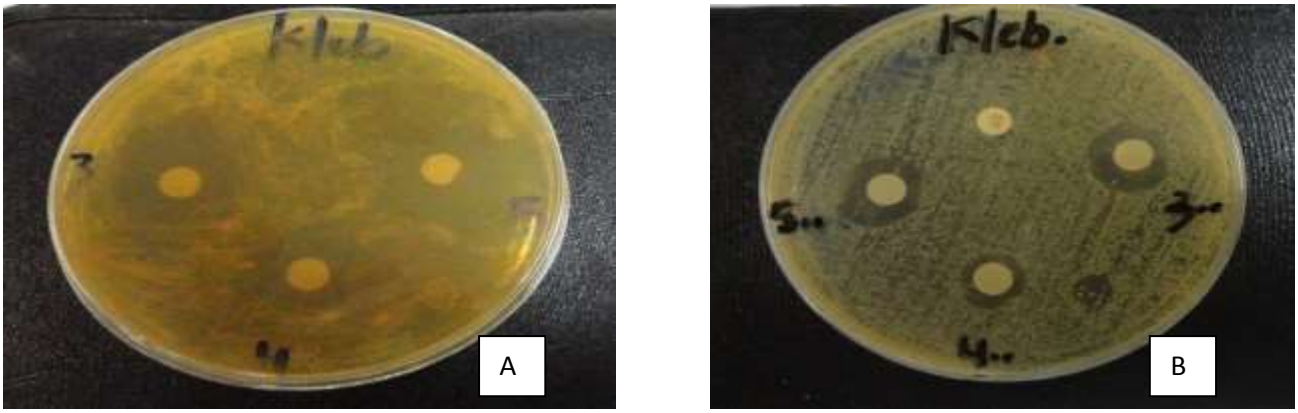


Figure 5: The inhibition zone in *Klebsella pneumonia* by Amoxicillin and Synthesized compound(300,400.500µg/ml). A; Amoxicillin. B; New compound

Conclusion

Amoxicillin is an excellent candidate to treat various infectious diseases but it is less effective against gram negative organisms, as well as bacterial resistance develop to the drug candidate, Where major development is required. In our study described the synthesis of novel derivative of amoxicillin drug, 6-({[(*Z*)-ethylidene amino] (4-hydroxyphenyl) acetyl} amino) -3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. The molecular structure of new compound was characterized by spectroscopic methods. The synthesized compound was investigated *in vivo* the toxic effects via the measurement of toxic dose (LD_{50}) which was moderate. Furthermore, the study included the in vitro antimicrobial, such as antibacterial activity against some bacterial. The synthesized compound showed antimicrobial activity against the tested organisms higher than those of the respective standard drug at tested doses level.

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