Pharmacological study of Schiff base derived from amoxicillin drug and vanillin

Huda K. Khasaf[1] and Wasfi A. Al-Masoudi [1]*

[1]University of Basrah, Faculty of Veterinary medicine, Department of Physiology, Pharmacology and Chemistry, Basrah, IRAQ

ABSTRACT

Condensation of 4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid,6-[[amino- (4-hydroxy phenyl) acetyl]amino]-3,3-dimethyl-7-oxo,trihydrate (Amoxicillin drug) with 4-Hydroxy-3-methoxy benzaldehyde yielded a novel Schiff base derivative of amoxicillin in good yield. A new compound was characterized by elemental analysis, IR and 1HNMR spectroscopy. The synthesized compound was screened for antibacterial activity against Staphylococcus aureus, Escherichia coli, Bacillus cereus, and Klebsella pneumonia. The toxicity of the compound was also assayed via the determination of their LD50 value by using Dixon,s up and down method . Studied compound was found to have an LD50 of 477.2 mg / kg of body weight.

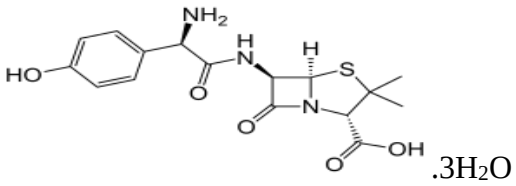
Key words: Amoxicillin, vanillin, Antibacterial activity, Schiff-base, Acute toxicity

***Correspondence:** E-mail: almasoudi59@yahoo.com, Tel: +9647809830756

INTRODUCTION

The β-lactams antibiotics are an important type of vital antibiotics used to treat infectious disease including tetracycleβ-lactam atoms (1).

Amoxicillin is a p-hydroxy derivative of ampicillin. The chemical name of amoxicillin (2)is: 4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, 6-[[amino (4-hydroxy phenyl) acetyl]amino]-3,3-dimethyl-7-oxo,trihydrate, ($C_{16}H_{19}O_5S$).$3H_2O$.



.$3H_2O$

Figure 1. Structural formula of amoxicillin trihydrate(AMX)

The bio-functional activity of AMX is related to lactam ring that inhibits bacterial growth by proteolysis mechanism (3). 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 (4,5).
Presence of amide and amino group in structure of amoxicillin suggest it a better starting compound for the synthesis of new Schiff base. Compounds containing an azomethine group (imine) are a class of important compounds in medicinal and pharmaceutical field (6).
Naz and Iqbal were synthesized some Schiff base complexes derived from amoxicillin which have good antibacterial activity (7). Al-Masoudi *etal*. found that the Schiff base derived from amoxicillin and 2-Hydroxy naphthaldehyde having good antibacterial activity when comparasion with control amoxicillin (8).
We describe here the synthesis of new amoxicillin derivative, invitro antibacterial and acute toxicity were evaluated.

EXPERIMENTAL

Materials

Infrared spectra (IR) were recorded as KBr discs in the range of 4000-400 cm-1using FT-IR spectrophotometer Shimadzu model IR. Affinity-1 at the Department of Chemistry, College of Education for pure sciences, University of Basrah, Iraq. 1H, 13C NMR spectra were measured on a Brucker at 600 MHz, with TMS as internal reference at Konstanz University, Germany. Microanalysis for carbon, hydrogen and nitrogen were carried out by a Perkin-Elmer 240B Elemental Analyzer. Melting points were measured by a Philip Harris melting point apparatus and uncorrected.

Antibacterial activity

The synthesized compound was screened invitro for their antibacterial activity against: Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Bacillus cereus, using the paper disc-agar diffusion technique on Muller Hinton agar as a culture media for antibacterial activity (9). The test compounds were dissolved in DMSO solvent and recommended concentrations (300,400 and 500µg/mL) were used in the disc-agar diffusion technique. Antibiotic drug Ampicillin were used as control.Petri plates containing 20 ml of Mueller Hinton Agar were used for all the bacteria tested. Sterile Whatman no. 1 filter paper disks (6mm in diameter) impregnated with dimethylsulfoxide (DMSO) of the test was placed on the Petri plates. A paper disk impregnated with dimethylsulfoxide (DMSO) was used as negative control. The plates were incubated for 24 h at 37°C in the case of bacteria. The inhibition zone diameters were measured in millimeters.

Acute toxicity (LD50)

All experiments were performed on 10-14/ weak old male and female rats, rats weighing 200-220gm at the time of treatment by using up-and-down method, Dixon,1980 (10).Males and females rats were injected intra peritoneal with different doses of the Amoxicillin derivative after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD50 were determined after reading final result (response-dead (X) or non response alive (O), then the following equation was applied LD50 = XF + Kd.
The estimate of LD50 is XF + Kd, where (XF) is the final test level and (K) is the interval between dose levels. (d) is the tabulated value as seen in Table 1.

Table1. Shows 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 :-				

LD50 =Xf+ Kd

LD50 = Median Lethal Dose, xf= Last dose used in the experiment,k =Factor of change from the table, d = Difference between doses.

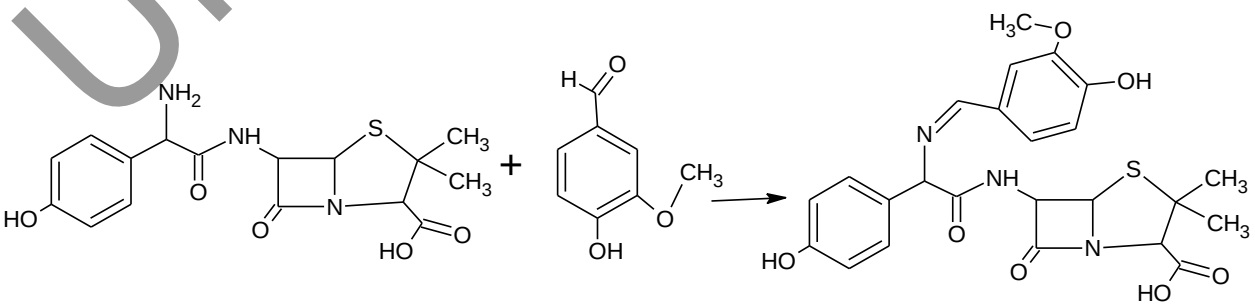
Synthesis and Characterization of Novel Schiff Base

A solution of Amoxicillin tri hydrate (2.247 gm, 5 mmole) in methanol (10 ml) was added to a solution of 4-Hydroxy-3-methoxybenzaldehyde (0.76 gm, 5mmole) in methanol (10 ml). The mixture was refluxed for (5 hours) with stirring. The resulting was an yellow solution allowed to cool and dried at room temperature, then re-crystallization to the precipitate with ethanol, pale-yellow solid was obtained by evaporation of ethanol during (24 hours) Scheme (1).

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,1H,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 $C_{24}H_{25}N_3O_7S$ (499.55): C, 57.65, H, 5.00, N, 8.40, Found: C, 57.92, H, 5.39, N, 8.71.



Scheme 1. Synthesis of the new Schiff base derived from amoxicillin drug.

RESULTS AND DISCUSSION:

Chemistry

Isolated yield, melting point, color and spectral data IR and 1H NMR of synthesized new compound was reported. The present work described the synthesis of new Schiff base derived from amoxicillin and aldehyde. Thus, reaction of 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 4-Hydroxy-4-methoxy benzaldehyde in 1:1 mole ratio gave the desired product in good yield. The IR spectrum of synthesized compound displayed common features in certain regions and characteristic bands in the fingerprint and other regions. The strong and broad bands in the rang 3362-3215cm-1 were assigned to ν(O-H) and ν(N-H) secondary amine stretching vibration with disappearance of the band for the ν (N–H) primary amine stretching vibration. The band at 1630cm-1 was due to azomethine group ν(-HC=N-) stretching vibrations, meanwhile the band at 1835 cm-1 was due to ν(C=O) cm-1 stretching vibration of CO2H group. The band at 1667 cm-1 stretching vibration was due to ν(C=O) for β-lactam group overlapping with ν(-HC=N-) stretching vibrations. The 1HNMR spectrum of synthesized compound showed that all the expected protons with proper intensity ratio. The singlets at and1.17 ppm were attributed to methyl protons and methoxy protons were appeared at. The aromatic protons of appeared in the region 7.38-6.71 ppm. The protons of hydroxyl groups of Ar-OH and -COOH were resonated as two singlets at 5.51 and 5.63 ppm, respectively. The proton of azomethine (CH=N) resonated as a singlet at8.51 ppm. Three groups of double peaks given by (CO–CH) and (N–CH) on the β-lactam ring and (NH sec.) amide appeared at 4.81,3.83 and 8.20 ppm, respectively.

Pharmacological Study

Median lethal dose (LD50)

Determination of the 50% of lethal dose (LD50) of the studied compound in- vivo was detected in the rats by using the "up-and-down" procedure described by (Dixon, 1980) (10). In the experiment we using 10 animals of white rats 10-14 weeks in age, Graded doses of injection to each one animal, a series of concentrations (150, 200, 250,300… 2500) mg/k.gb.w) in 0.1 ml (Dimethylsulphoxide) DMSO, were administered and chosen with equal spacing (concentrations) between doses. Mortality was recorded after 24 hrs that each one animal treated with one dose and after 24 hrs was recorded as O if the animal lives and then increased the treated dose. While X recorded for the death of animal and then decreased the dose according for the result of the animal the code which formed as being (OOOX) and according for Dixon value was get and the LD50 was determined according to the formula employed by Dixon (1980).

LD50 =Xf+ K
LD50 = 400 + (1.544) x50
LD50 = 477.2 mg / kg b.w

Antibacterial activity:

The antibacterial activity results are shown in Table 2, The amoxicillin compound show no activity against *Escherichia coli, staphylococcus* sp. Figures 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, Figure 3.

Table 2. Antibacterial activity of amoxicillin and new compound.

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

The new compound as showing by Figure 4 have a moderate activity against *Klebsella* sp. while the amoxicillin derivative shows high degree of activity at all concentration.

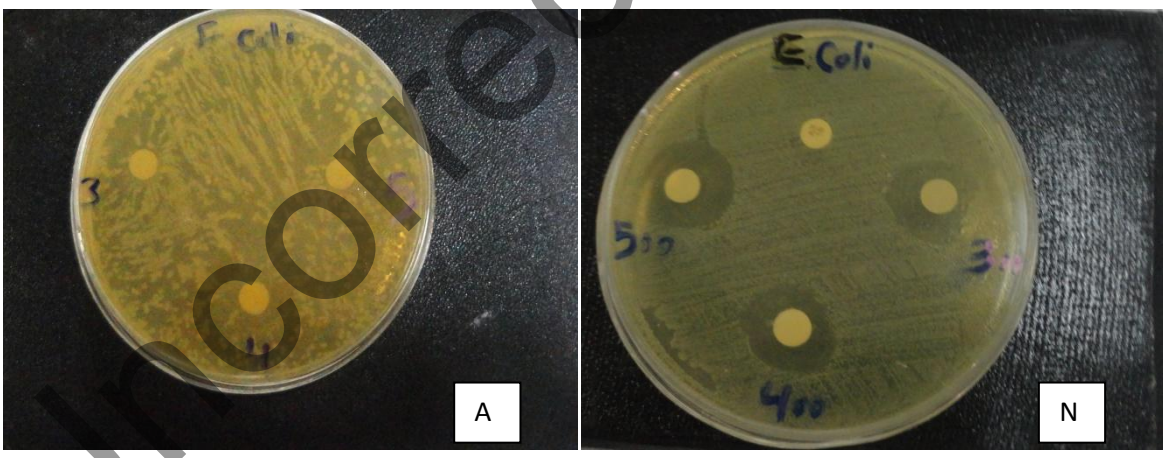


Figure 2. Show the inhibition zone in *E. Coli* by Amoxicillin and Synthesized compound (300,400.500µg/ml). A= Amoxicillin, N= New compound

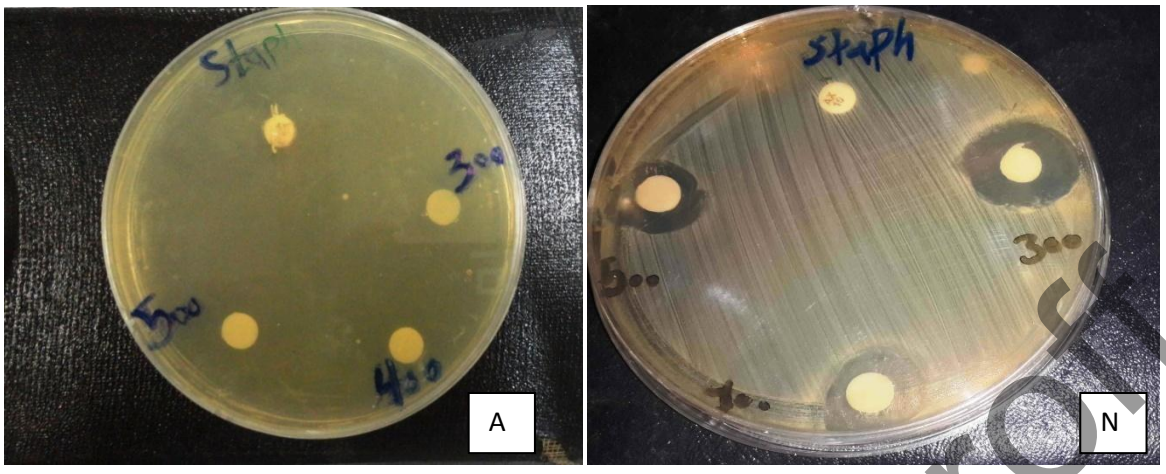


Figure 2. Show the inhibition zone in *Staphylococcus aureus* by Amoxicillin and Synthesized compound(300,400.500µg/ml). A= Amoxicillin, N= New compound.

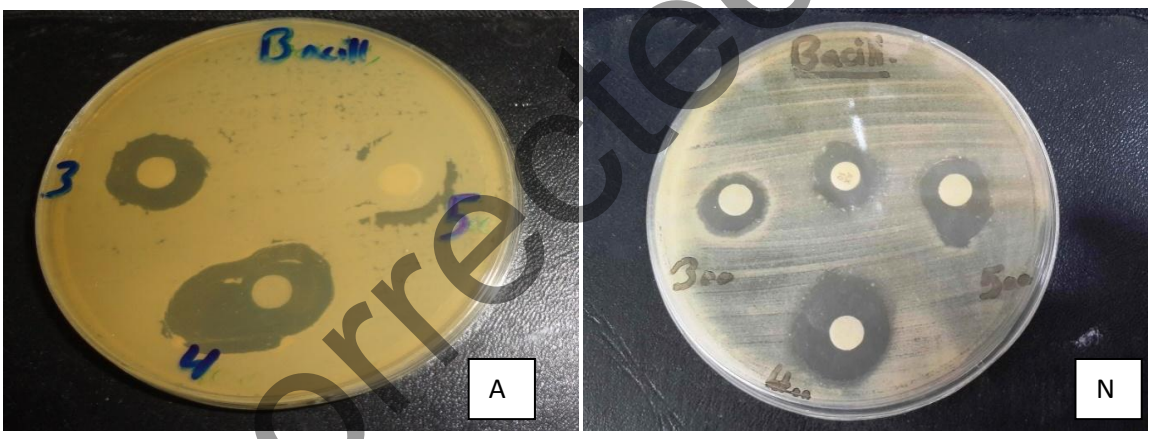


Figure 4. Show the inhibition zone in *Bacillus. aureus* by Amoxicillin and Synthesized compound (300,400.500µg/ml). A= Amoxicillin, N= New compound.

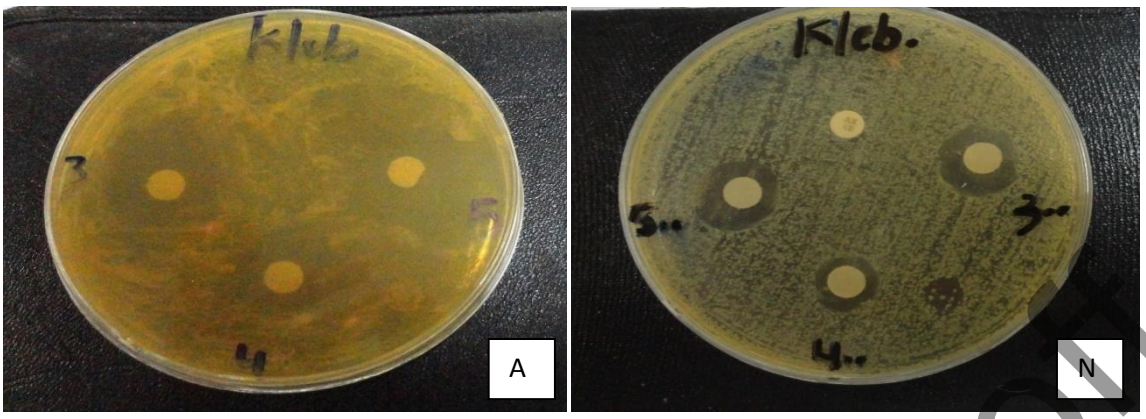


Figure 5. Show the inhibition zone in *Klebsella pneumonia* by Amoxicillin and Synthesized compound(300,400.500µg/ml). A= Amoxicillin, N= New compound.

CONCLUSION

In conclusion, the present study described the synthesis of novel derivative of amoxicillin Drug,6-({[(Z)-ethylideneamino](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 (LD50) 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.

ACKNOWLEDGMENT

The authors are grateful to Prof.Dr. Yassin Al-Soud (Al-Baiet University, Jordan) for providing of some elemental analysis and NMR spectroscopy. We are also grateful to Departments of Physiology and Microbiology, College of Veterinary Medicine, Al-Basrah University, Iraq for providing the facilities.

Note: Ethical standards have been followed in accordance with the instructions of the Laboratory Animal Handling Committee at the Faculty of Veterinary Medicine, University of Basrah

REFERENCES

1. Krik and othmer, "Encyclopedia of chemical technology", 4th ed., John wiley& Sons Inc., New York. 1992; Vol.3: p.1-250
2. Newton G.G.F. and AbrahamE.P.Isolation of cephalosporin C, a penicillin-like antibiotic containing d-a-aminoadipic acid, Biochem. J.,1956; 62: 651.
3.Zayed M.Abdallah S.Synthesis and structure investigation of the antibiotic amoxicillin complexes of d-block elements, SpectrochimicaActa Part A; Molecular and Biomolecullar spectroscopy, 2005;61(9):2231-2238.
4. Kuar S.P., Rao R. and Nanda S. Amoxicillin: A broad spectrum antibiotic, International Journal of Pharmacy and Pharmaceutical Sciences, 2011; (3), 14-19.
5.SinghK.,Barwa M., TyagiP.Synthesis and characterization of cobalt(II), nickel(II), copper(II) and zinc(II) complexes with Schiff base derived from 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine,Eur. J. Med. Chem.2007;42: 394- 402.

6. Bagihalli, G. B.; Avaji, P. G.; Patil, S. A.; Badami, S. Synthesis, spectra characterization, in vitro anti bacterial, antifungal and cytotoxic activities of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole Schiff bases Eur. J. Med. Chem.2008;43: 2639- 2649.
7. Naz, N. and IqbalM. Z.Synthesis, Spectroscopic and biological studies of transition metal complexes of novel Schiff bases derived from amoxicillin and sugars.J. Chem. Soc. Pak., 2009; 31:440-446.
8. Al-Masoudi W. A., Mohammad, H. T., Hama A. A., Synthesis, characterization and pharmacological study of new Schiff base derived from amoxicillin drug.
9. Wayne, (National Committee for Clinical Laboratory Standard, NCCLS Approved standard M27-PA), 1997, USA.
10. Dixon, W. J. Efficient analysis of experimental observations, Ann. Rev. Toxicol., 1980; 20, 441-462.