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Research Article

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL STUDY OF NEW SCHIFF BASE DERIVED FROM AMOXICILLIN DRUG

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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 with 2-hydroxy-1- naphthaldehyde yielded a novel Schiff base derivative of amoxicillin in good yield. A new compound was characterized by elemental analysis, IR, and ^{1}H-NMR spectroscopy. The synthesized compound was screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus, Streptococcus, Klebsella, salmonella* and *Psedumonas spp* and fungicidal activity against *Aspergillus multi, Aspergillus niger*, *Candida albicans, Candida trobicalis and Candida krusi*. The toxicity of the compound was also assayed via the determination of their LD_{50} value by using Dixon's up and down method (1980). Studied compound was found to have an LD_{50} of 492.8 mg / kg of body weight.

Keywords: Amoxicillin, 2-hydroxy-1- naphthaldehyde, Microbial activity, NMR spectroscopy, Acute toxicity.

INTRODUCTION

Antibiotics are chemical substances produced by various species of microorganisms and other living systems that are capable in small concentration inhibiting the growth of or killing bacteria and other microorganisms.
The β-lactams antibiotics are an important type of vital antibiotics used to treat infectious disease including tetracycle β-lactam atoms[1].

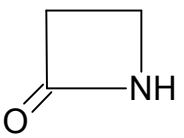
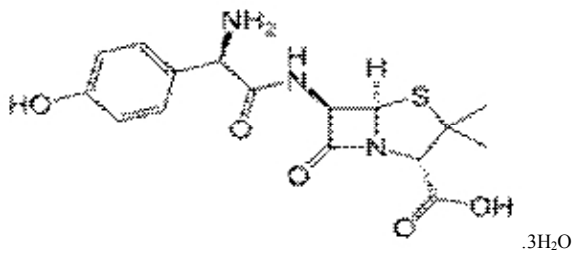


Figure 1: Structure of β-Lactam

The β-lactam antibiotics inhibit bacteria, exhibiting activities that differ in pattern and intensity[2].

Amoxicillin is a p-hydroxy derivative of ampicillin. The chemical name of amoxicillin[3] 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 2: Structural formula of amoxicillin trihydrate

Amoxicillin is a bacteriolytic β-lactam antibiotic drug of class penicillin, active against various gram positive and gram negative bacteria[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.

Some Schiff-bases were exhibits antibiotic, antiviral and antitumor agents because of their specific structure. 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[6].

Naz and Iqbal[7] found that the Schiff base complexes derived from amoxicillin having good antibacterial activity in good range when comparison to control (Amoxicillin).

In view of the importance of such imines, we describe here the synthesis, characterization, *invitro* anti microbial activity and acute toxicity of novel Schiff base by reaction of amoxicillin trihydrate drug with 2-hydroxy-1- naphthaldehyde.

MATERIALS AND METHODS

Physical measurements

Infrared spectra (IR) were recorded as KBr discs in the range of 4000-400 cm^{-1} using FT-IR spectrophotometer Shimadzu model IR. Affinity-1 at the department of Chemistry, College of Education for pure sciences, University of Basrah, Iraq. ^{1}H 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 point was measured by a Philip Harris melting point apparatus and uncorrected.

Antimicrobial activity

The *invitro* biological screening of the 4-[(E)-(3,4-dihydroxybenzylidene)amino]-N-(1,3-thiazol-2-yl) benzenesulfonamide was investigated against various bacterial species like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus, Streptococcu , Klebsella, salmonella* and *Psedumonas sp* and fungicidal activity against *Aspergillus multi, Aspergillus niger*, *Candida albicans, Candida trobicalis and Candida krusi* using the disc-agar diffusion technique[8]. Muller Hinton agar was used as culture media for antibacterial activity. The antifungal activities were tested against fungus above by disk diffusion method. Recommended concentration 50, 100 and 200 µg/ml of the test samples in DMSO solvent was introduced in the respective method. Antibiotic drugs Amoxicillin (10 mg) were used as control for bacteria and fungi. Petri plates containing 20 ml of Mueller Hinton Agar were used for all the bacteria tested. *Aspergillus multi, Aspergillus niger*, *Candida albicans*, *Candida trobicalis and Candida krusi* strains was cultivated in Sabouraud's dextrose agar. Sterile Whatman no.1 filter paper disks (6mm in diameter) impregnated with the solution in DMSO of the test were placed on the Petri plates. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. The plates were incubated for 24 h in the case of bacteria and 72 h for fungi at 28 °C. The inhibition zone diameters were measured in millimeters using a caliper vernia.

Acute toxicity (LD50)

Animals. All experiments were performed on 10-14- weak old male and female ratus-ratus/rats weighing 200-250 gm at the time of treatment by using up-and-down method, Dixon[9] 1980. Male and female rats were injected intraperitonially 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. LD_{50} were determined after reading final result (response-dead (X) or non response alive (O) , then the following equation was applied $LD_{50} = XF + Kd$.

The estimate of LD_{50} is XF + Kd, where (XF) is the final test level and (K) is the interval between dose levels. (d) is the tabulated value (Table 1).

Table 1: Dixon values; Dixon (1980)

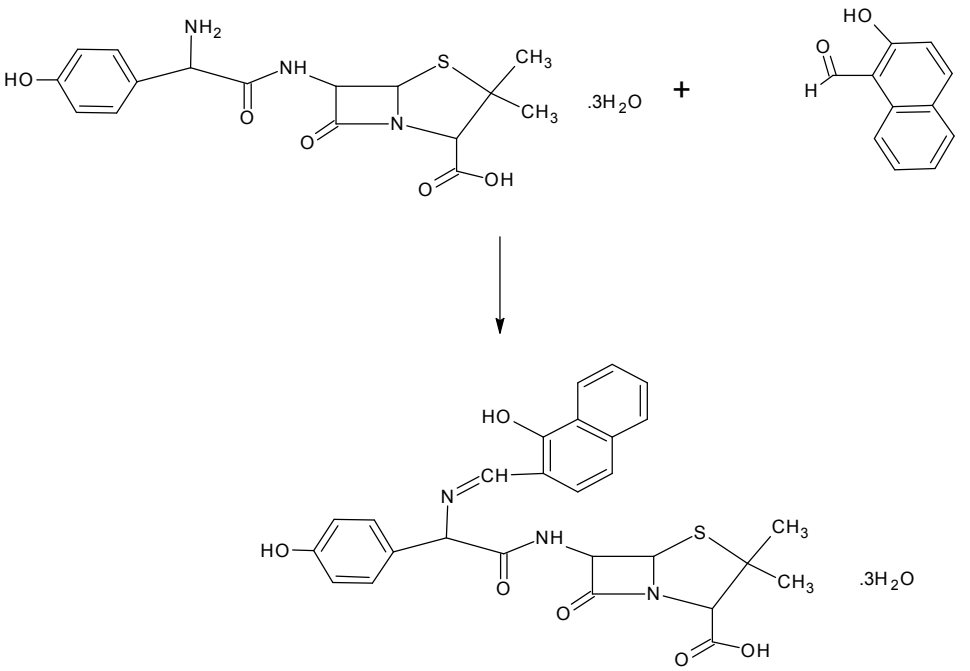
	K represented serial tests started with :-				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544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

LD_{50} = Xf+Kd
LD_{50} = 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 Amoxicilline trihydrate (2.247 gm, 5 mmole) in methanol (10 ml) was added to a solution of 2-Hydroxy-1-naphthaledyne (0.870 gm, 5mmole) in methanol (10 ml). The mixture was refluxed for (5 hours) with stirring. The resulting was 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; 82%, M.P.= 137-140^{0}C. FT-IR(KBr, cm^{-1}), 310-3200(OH, NH); 3068(C-H, aromatic); 2968,2933(C-H,aliphatic);1850(C=OCarboxylic);1660(C=O,β-Lactam);1635(C=N);1616(C=C);
1597, 1369 (COOH).
^{1}H NMR(DMSO-d_6); δ 9.42(s, 1H, COOH); 8.36(s,1H,CH=N); 8.10(s, 1H,NH amide); 7.10-6.71(m,10H-Ar-H);5.57,5.68(s,2H-Ar-OH); 4.6(s,1H-CH-N),4.7(s,1H,CH-COOH) , 3.34(1H,CH-S), 1.50,1.24(s,6H-2CH_3).
Anal. for $C_{27}H_{25}N_3O_6S.3H_2O$(M.wt 573): Calc. C, 56.54; H, 5.41; N, 7.32; Found: C,56.58; H,
4.78 ; N, 7.31.



Scheme 1: Synthesis of new Schiff base derived from amoxicillin drug

RESULTS AND DISCUSSION

Chemistry

Isolated yields, melting points, colors and spectral data IR and ^{1}H NMR of synthesized novel compound was reported. The present work describes the synthesis of new Schiff base derived from amoxicillin and aldehyde to produce bioactive Schiff base, thus, the 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 2-hydroxy naphthaldehyde in 1:1 mole ratio gave the new organic compound in good yield. IR spectra for synthesized compound displayed common features in certain regions and characteristic bands in the fingerprint and other regions. The IR spectra of new prepared compound show strong and broad bands in the rang 3310-3200 cm-^{1} due to ν(O-H) and ν(N-H) secondary amine stretching vibration and disappeared the band for the ν (N–H) primary amine stretching vibration. The IR spectra of synthesized compound displays band at 1635cm^{-1} is due to azomethine group ν (-HC=N-) stretching vibrations. The band at 1850 cm^{-1} is due to ν (C=O) cm^{-1} stretching vibration for (COOH). The band at 1660 cm^{-1} stretching vibration is due to ν (C=O) for β-Lactam group overlapping with ν (-HC=N-) stretching vibrations. The bands at 1597 cm^{-1}, and 1369 cm^{-1} were assigned to stretching vibration (COOH) asymmetric and symmetric stretching vibration, respectively. The bands at 1616, 3068, 2968-2933 cm^{-1} were assigned to ν (C=C), aromatic, ν (C–H) aromatic and ν (C–H) aliphatic, respectively[10].

The ^{1}H NMR spectra of studied synthesized compound was recorded in DMSOd_6 solution and show all the expected protons with proper intensity ratio. In ^{1}H NMR spectrum of the synthesized compound in DMSO-*d6*, single peaks attributed to methyl groups appeared at 1.50 and 1.24 ppm ($2CH_3$). The aromatic protons of compound appeared within the range 7.10-6.71 ppm. It is worthy to note that the protons of Ar-OH resonate as a singlet at 5.68 and 5.57 ppm. The proton of azomethine (CH=N) resonate as a singlet at 8.36 ppm, single peak attributed to hydroxyl group of carboxylic appeared at 9.42 ppm. Three groups of double peaks given by (CO–CH) and (N–CH) on the β-Lactam ring and (NH sec.) amide appeared at 4.6, 4.7 and 8.10 ppm, respectively[11]. ^{1}H NMR show signal at 3.34 ppm attributed to (CH-S) group.

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)[9]. 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 (250, 300, 350, 400,450 and 500) mg/k.g b.w) in 0.1 ml (Dimethyl sulphoxide) 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 (OOXO) and according for Dixon value was get and the LD50 was determined according to the formula employed by Dixon (1980).

LD50 =X*f* + Kd
LD50 = 500 + (-0.144) x 50
LD50 = 492.8 mg / kg b.w
1/10 LD50= 49.28 mg / kg (1 kg = 6 rats) Depending on the weight rat about 175 gram)**.**
1/10 LD50= 8.213 mg /rat Depending on the weight rat 175 gram.

Microbial study

The results of the antibacterial activity are shown in Table 2. The studied compound show no activity against *Escherichia coli*, *Streptococcus sp and Psedumonas sp* but moderate active in *Bacillus cereus* and *Staphylococcus aureus* at all concentrations. The new compound show high activity against *Salmonella sp* and *Klebsella sp.* The results of antifungal activity of the compound show not active towards *Candida trobicalis, Candida krusi and Aspergillus niger* but moderate active against *Candida albicans* and *Aspergillus multi* at 200 µg/ml compared with amoxicillin, Table 3.

The bacteria and fungi were supplied from department of Microbiology, College of Veterinary Medicine, University of Basrah.

Table 2: Antibacterial activity of the Schiff-base derivatives of amoxicillin drug

Comp.	Conc. µg/ml	*E.coli*			*B.cerus*			*Strepto.*			*Staph.*			*Salmonella*			*Klebsella*			*Psedumonas*		
		50	100	200	50	100	200	50	100	200	50	100	200	50	100	200	50	100	200	50	100	200
Amoxicillin		-	-	-	7	7	7	-	-	-	7	8	8	10	15	15	15	20	25	-	-	-
New compound		-	-	-	8	8	8	-	-	-	8	9	9	15	15	15	10	10	12	-	-	-

Diameter of inhibition zone in mm for different microbial species

Table 3: Antifungal activity of the Schiff-base derivatives of amoxicillin drug

Comp.	Conc. µg/ml	*C.trobicalis*			*C.krusii*			*A.multii*			*C.albicans*			*A.niger*		
		50	100	200	50	100	200	50	100	200	50	100	200	50	100	200
Amoxicillin		-	-	-	-	-	-	-	10	12	10	15	18	-	-	-
New compound		-	-	-	-	-	-	-	-	8	7	8	8	-	-	-

Diameter of inhibition zone in mm for different microbial species

CONCLUSION

In conclusion the present study was, firstly, to synthesis of novel derivative of amoxicillin Drug named 4-[(E)-(3,4-dihydroxybenzylidene)amino]-N-(1,3-thiazol-2yl)benzenesulfonamide. The molecular structure of new compound was characterized by spectroscopic methods. The Synthesized compound was investigated in vivo toxic effects and to find acute toxic dose (LD_{50}) which have moderate toxicity. And secondly, to investigate in vitro antimicrobial activity, such as, antibacterial and anti fungal activity against some bacterial and fungi in hope to expansion their biological studies in future.

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