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Synthesis, Characterization and Antibacterial Studies of 2-azetidinones Compounds Derived from Amoxicillin

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Abstract:

In this study, the new azetidinones were synthesized from Schiff bases 2(a-j) that derived from amoxicillin (1) on treatment with chloroacetyl chloride in presence of triethylamine gave azetidinone 3(a-j). The structure of these compounds have been elucidated on the basis of their physical and spectral. Azetidinone compounds were also screened for their antibacterial activity against some bacterial species using amoxicillin as standard.

***Keyword**: Schiff bases, azetidinones, synthesis, antibacterial activity.*

تحضير وتشخيص ودراسة الفعالية المضادة للبكتريا لمركبات ٢- ازيتيدينونات المشتقة من الاموكسيسلين

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*****فرع الكيمياء الصيدلانية ،كلية الصيدلة،الجامعة المستنصرية**

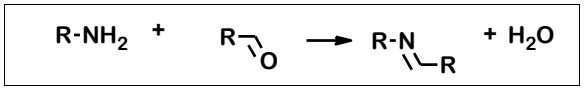
الخلاصة:

حضرت مركبات جديدة للازيتدينونات من قواعد شف المحضرة من الاموكسيسلين بمعاملتها مع كلورواستيل كلورايد بوجود ثلاثي اثيل امين ليعطي مركبات الازيتدينون. تراكيب المركبات المحضرة تم تشخيصها من خلال خواصها الفيزياوية والطيفية كما تم قياس الفعالية البايولوجية لمركبات الازيتدينون ضد بعض الانواع البكتيرية باستخدام الاموكسيسلين كمقياس.

Introduction:

Schiff bases are very important compounds and are being used in various fields. Schiff bases derived from the amine and carbonyl compounds have various applications in different fields including biological, inorganic and analytical Chemistry[1-6].

Schiff bases are normally produced by the condensation of primary amine and aldehyde or ketone. The consequential compound is called a Schiff bases, named after the scientist who synthesized it first [7].



Here R may be an aliphatic or aromatic group. Amoxicillin trihydrate is effect via oral route in the case of sensitive gram-positive and gram-negative organisms. IUPAC name of amoxicillin is 6-{[amino(4-hydroxyphenyl)acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. The structure formula is given below:

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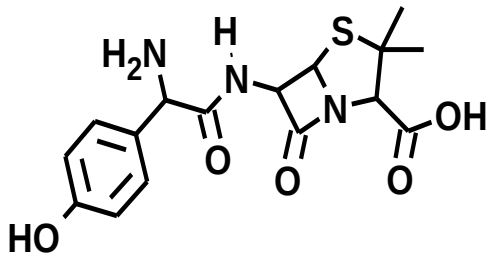

2-azetidinone, commonly known as beta-lactams, are well-known heterocyclic compounds a many the organic and medicinal chemists[8]. The activity of famous antibiotics such as Penicillins, Cephalosporins and Carbapenems are attributed to the presence of 2-azetidinone ring in them. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing 2-azetidinone ring[9]. Such biological activities include antifungal, antitubercular, antitumor, cholesterol absorption inhibition and enzyme inhibition activity[10] . The beta-lactam also serve as synthons for many biologically important classes of organic compounds . The long-term use of beta-lactam antibiotics exerts selective pressure on bacteria and permits the proliferation of resistant organisms[11]. . Azetidinones, which are part of antibiotic structure, are known to exhibit interesting biological activities.

Materials and Methods:

All chemicals used were of analytical reagent grade and they were available from Aldrich and Fluka Companies and amoxicilline trihydrate standard material was provided from state company for drug industries and medical appliance - (SDI) Samaraa – Iraq.

Melting points were determined in an open capillary tube and are uncorrected. Infrared spectra were recorded in KBr on Shimadzu spectrophotometer. The ^{1}HNMR were measured in DMSO solutions on a Bruker-400 MHz spectrometer using TMS as internal reference(chemical shift in ppm). All reactions was monitored by thin layer chromatography (TLC) and spots were visualized using iodine chamber. The antibacterial activity was determined by Agar-well diffusion method.

Synthesis Schiff bases 2(a-j)[12].

Amoxicillin trihydrate (3 mmol, 1.1622 g) dissolved in methanol (30 mL) was mixed with different aldehydes and keton (3 mmol) dissolved in methanol (30 mL). To this KOH alcoholic (0.1% methanol) was added to adjust the pH of the solution between 6-7 and the mixture was refluxed for 4-6 hr (approx.). A clear colored solution was obtained. The Schiff base was isolated by crystallization after volume reduction by evaporation. The crystalline product was dried under vacuum and kept in desicator till further use.

Synthesis beta-lactam derivatives 3(a-j) [13].

Chloroacetyl chloride(4 mmol) was added to Schiff base (2 mmol) and triethyl amine dissolved in dry 1,2-dioxane (30 mL)at10 °C. The mixture was stirred for 24 hr. The triethyl amine hydrochloride precipitate formed was filtered and washed several time with dry 1,4-dioxane. The filterate and washing were mixed and concentrated under reduced pressure the residue was poured into crushed ice and the oily product obtained was recrystallized from ethanol.

Results and Discussion:

In the present work, series of Schiff bases were synthesized from amoxicillin (1) with different aldehydes and ketones. The resulted Schiff bases 2(a-j) undergo cyclization reaction with chloroacetyl chloride in presence of triethylamine under cold condition will yields the beta- lactams compounds 3(a-j) .Scheme 1 outline the synthetic sequences for preparation of Schiff base compounds 2(a-j) and beta-lactam compounds 3(a-j).

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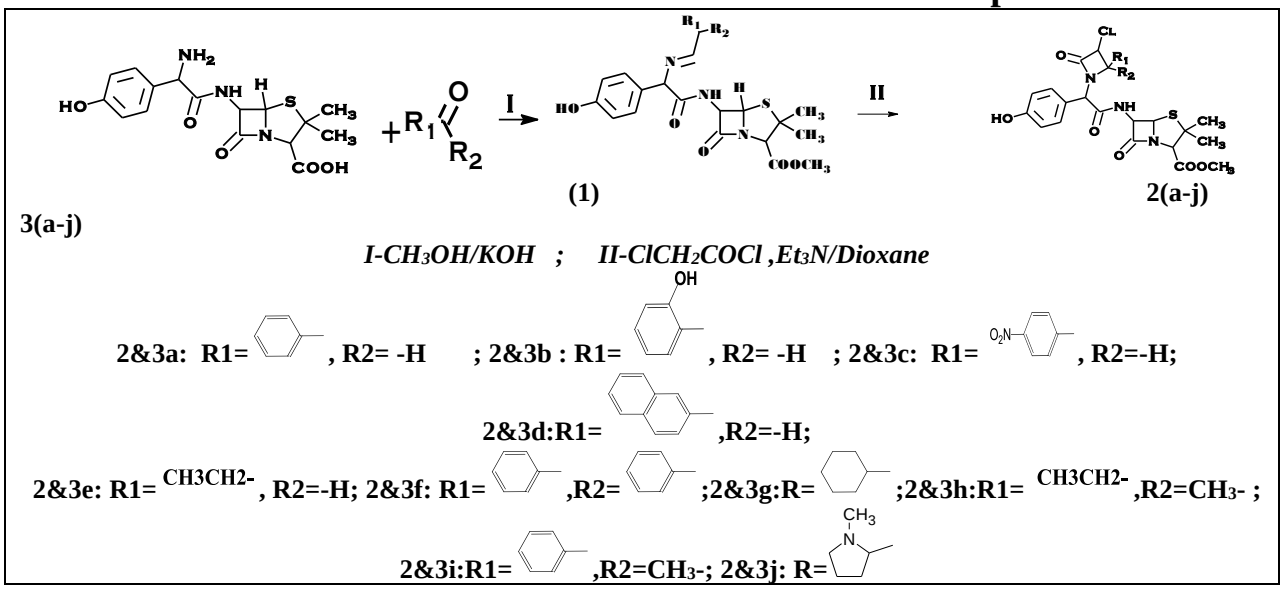

Scheme-1: Preparation of synthesized compounds.

The structural assignment of amoxicillin trihydrate was based on melting point (182ºC decomposed) and FT-IR spectrum for amoxicillin exhibits two bands at (3171 and 3464 $cm^{-1)}$ for–NH_2 group besides this bands about (1766 and 1687 $cm^{-1)}$ due to C=O for lactam and carboxyl group, the band appeared at (3464 $cm^{-1)}$ was assigned to the hydroxyl group H-O .

The Schiff bases derivatives of amoxicillin were prepared in good yield. The physical properties and FT-IR spectral of Schiff bases compounds listed in table-1. The FT-IR spectra of Schiff bases 2(a-j) indicated the presence of CH=N (1597-1626 cm^{-1}) and disappearances two bands at (3342 cm-1) and (3171 cm^{-1}) for asymmetric and symmetric stretching vibration of NH_2 group of amoxicillin.

The azetidinone compounds 3(a-j) that prepared from corresponded Schiff bases in low yield.

The physical properties and FT-IR spectral data of listed in table-2. The FT-IR spectra of beta lactams 3(a-j) appeared band in the region (693-720 $cm^{-1)}$ for C-Cl and disappearance band of –N=CH-group (1597-1626cm^{-1}).

^{1}H NMR spectra data (δ ppm) in DMSO-d_6 solvent of compounds 2a and 2b showed a singlet at δ 8.21 and 8.12 ppm due to the imines CH=N- protons, and signal at δ 3.69 and δ 3.66 ppm due to –COO$\underline{CH_3}$ protons respectively.

^{1}H NMR of compound 3b appeared a signal at δ 5.23 ppm for compound 3b due to -$\underline{CH}$-Cl and N-$\underline{CH}$-CH-Cl for azetidinone protons and disappearance the signal at δ 8.21 ppm due to proton CH=N- in 2b. The 1H NMR data for compound 2a, 2b and 3b listed in table-3, figures 1,2 and 3 .

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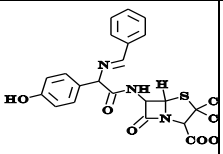
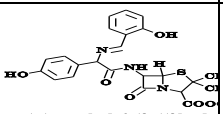
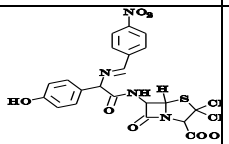
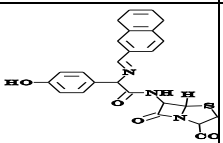
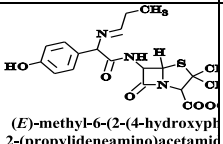
Table-1: Physical properties and FT-IR spectral data of the prepared compounds 2(a-j).

Cop. Code	Physical properties				Major FT-IR absorption cm^{-1}					
	Structure	Dec.p ^{O}C	Yied %	Color	ν(N-H)	ν(C-H) aliph.	ν C-H) arom.	ν(C=C) arom.	ν(CH)	ν (C=O) 1, 2& 3
2a	 (*E*)-methyl 6-(2-(benzylidene 2-(4-hydroxyphenyl)acetamid dimethyl-7-oxo-4-thia-azab [3.2.0]heptane-2-carboxy	**198**	92	yellow	3333	2968 2946	3042 3033	1597 1516	1610	1.Azetidin one 1757 2.Amide 1661 3.Ester 1738
2b	 (*E*)-methyl 6-(2-((2hydr benzylidene)amino)-2-(4- roxyphenyl)acetamido)-3, methyl-7-oxo-4-thia-1azab [3.2.0]heptane-2-carboxy	**190**	88	Yellow	3316	2965 2890	3040 3025	1596 1514	1626	1.Azetidin one 1766 2.Amide 1663 3.Ester 1738
2c	 (*E*)-methyl 6-(2-(4-hydroxy 2-((4-nitrobenzylidene)amin amido)-3,3-dimethyl-7-oxo- azabicyclo[3.2.0]heptane-2car	**170**	86	Deep orange	3333	2967 2932	3056 3034	1588 1518	1605	Azetidino ne 1756 Amide 1647 3.Ester 1739
2d	 (*E*)-methyl 6-(2-(((5,8-dihydr thalen-2-yl)methylene)amino hydroxyphenyl)acetamido)- methyl -7-oxo-4-thia-1-azabi [3.2.0]heptane-2-carboxyl	175	89	yellow	3273	2965 2928	3082 3036	1590 1514	1613	1.Azetidin one 1763 2.Amide 1665 3.Ester 1736
2e	 (*E*)-methyl-6-(2-(4-hydroxypl 2-(propylideneamino)acetamid dimethyl-7-oxo-4-thia-1-azab [3.2.0]heptane-2-carboxyl	210	81	Pale yellow	3304	2967 2940	3044	1597 1514	1620	1.Azetidin one 1759 2.Amide 1670 3.Ester 1738

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Continue Table-1:

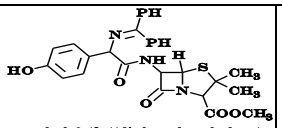
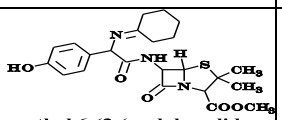
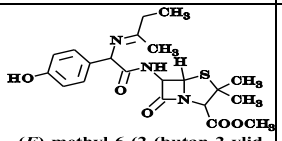
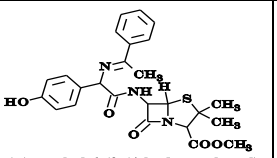
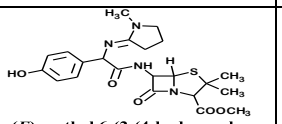
2f	 methyl 6-(2-((diphenylmethylene) amino)-2-(4-hydroxyphenyl)acet amido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2carboxylate	195	71	yellow	3275	2964 2928	3040 3022	1582 1512	1597	1.Azetidinone 1760 2.Amide 1661 3.Ester 1738
2g	 methyl 6-(2-(cyclohexylidene amino)-2-(4-hydroxyphenyl) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylate	212	88	yellow	3310	2934 2866	3042	1596 1515	1661	1.Azetidinone 1761 2.Amide 1676 3.Ester 1741
2h	 (*E*)-methyl-6-(2-(butan-2-ylid eneamino)-2-(4-hydroxyphenyl) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate	202	92	Pale yellow	3302	2966 2929	3036	1598 1514	1613	1.Azetidinone 1756 2.Amide 1667 3.Ester 1734
2i	 (*E*)-methyl 6-(2-(4-hydroxyphenyl)-2-((1-phenylethylidene)amino)acet amido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate	190	81	yellow	3268	2994 2976	3057 3033	1586 1516	1612	1.Azetidinone 1757 2.Amide 1640 3.Ester 1741
2j	 (*E*)-methyl 6-(2-(4-hydroxyphen yl)-2-((1-methylpyrrolidin-2-yl idene)amino)acetamido)-3,3-di methyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylate	174	83	yellow	3290	2964 2930	3060	1597 1514	1622	1.Azetidinone 1755 2.Amide 1661 3.Ester 1736

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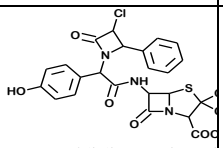
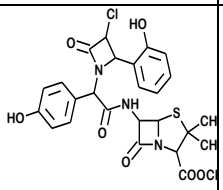
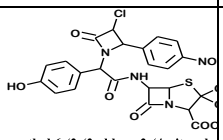
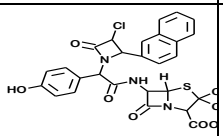
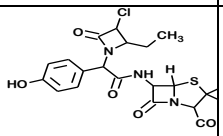
Table-2: Physical properties and FT-IR spectral data of azetidinone the compounds 3(a-j).

Comp. Code	Physical properties				Major FT-IR absorption cm^{-1}					
	Structure	Dec.p ^{O}C	Yield %	Color	ν (N-H)	ν (C-H) aliph.	ν (C-H) arom.	ν(C=C) arom.	ν(CCl)	ν(C=O) 1,2 & 3
3a	 methyl 6-(2-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate	Oily	56	brown	3306	2967 2933	3062 3025	1597 1516	700	1.Azetidinone 1760 2.Amide 1597 3.Ester 1734
3b	 methyl 6-(2-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(4-hydroxy phenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxy	Oily	50	brown	3331	2961 2924	3077 3030	1600 1514	700	1.Azetidinone 1758 2.Amide 1670 3.Ester 1738
3c	 methyl 6-(2-(3-chloro-2-(4-nitrophe 4-oxoazetidin-1-yl)-2-(4-hydroxyph)acetamido)-3,3-dimethyl-7-oxo-4-th azabicyclo[3.2.0]heptane-2-carboxy	Oily	46	brown	3290	2969 2926	3094 3034	1613 1514	696	1.Azetidinone 1754 Amide 1655 3.Ester 1734
3d	 methyl 6-(2-(3-chloro-2-(naphthalen-2-yl)-4-oxoazetidin-yl)-2-(4-hydroxyphenyl)acetam 3,3-dimethyl-7-oxo-4-thia-1-az cyclo[3.2.0]heptane-2-carboxy	Oily	55	brown	3256	2961 2896	3059 3018	1596 1516	710	1.Azetidinone 1758 2.Amide 1688 3.Ester 1740
3e	 methyl 6-(2-(3-chloro-2-ethyl-4-oxoazetidin-1-yl)-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbox	Oily	52	brown	3250	2990 2928	3066	1614 1516	704	1.Azetidinone 1759 2.Amide 1668 3.Ester 1734

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Continue Table-2

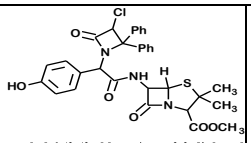
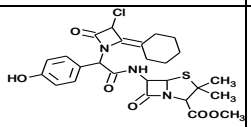
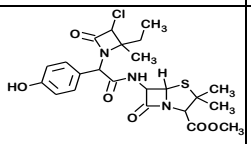
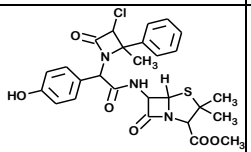
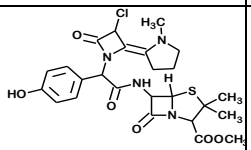
3f	 methyl 6-(2-(3-chloro-4-oxo-2,2-diphenyl azetidin-1-yl)-2-(4-hydroxyphenyl) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate	Oily	59	brown	3298	2969 2934	3059 3032	1601 1514	704	1.Azetidinone 1762 2.Amide 1657 3.Ester 1741
3g	 methyl 6-(2-(3-chloro-2-cyclohexylidene-4-oxoazetidin-1-yl)-2-(4-hydroxyphenyl) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate	Oily	69	brown	3296	2961 2932	3070	1616 1516	720	1.Azetidinone 1760 2.Amide 1668 3.Ester 1742
3h	 methyl 6-(2-(3-chloro-2-ethyl-2-methyl-4-oxoazetidin-1-yl)-2-(4-hydroxyphenyl) acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate	Oily	48	brown	3287	2967 2932	3073	1586 1514	702	1.Azetidinone 1761 2.Amide 1655 3.Ester 1740
3i	 methyl 6-(2-(3-chloro-2-methyl-4-oxo-2-phenylazetidin-1-yl)-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicycl[3.2.0]heptane-2-carboxylate	Oily	57	brown	3296	2965 2938	3088 3042	1590 1516	693	1.Azetidinone 1762 2.Amide 1670 3.Ester 1738
3j	 methyl 6-(2-(3-chloro-2-methyl-4-oxo-2-phenylazetidin-1-yl)-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate	Oily	56	brown	3289	2965 2932	3084 3038	1614 1514	698	1.Azetidinone 1758 2.Amide 1665 3.Ester 1739

Table-3: ^{1}HNMR spectral data (δ ppm) for selected compounds.

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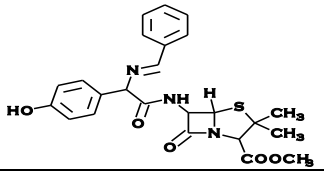
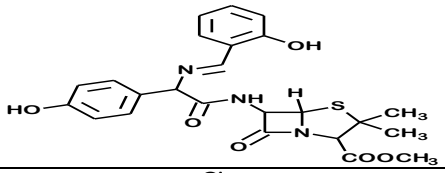
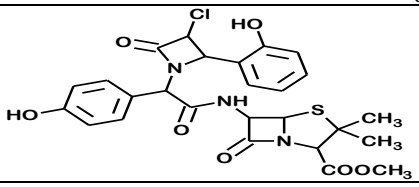
Comp. Code	Structure	^{1}HNMR Spectral data (δ ppm)
2a		6.7-7.23 (m,9H,Ar-H); 8.21 (s,1H,CH=N); 3.69(s,3H,-COOCH3);5.38(s,1H,O-H phenolic) 8(s1H,NH-amide); 5.41(s,-CH-N=CH-); 1.55(s,6H,-CH3); 4.89(d,-CH-CH-N-)
2b		6.71-7.42 (m,8H,Ar-H); 8.12 (s, H, CH=N); 3.66 (s,3H,-COOCH3); 5.67(s,1H,O-H phenolic); 8.03(s1H,NH-amide); 5.36 (s,-CH-N=CH-); 1.51(s,6H,-CH3); 4.86(d,-CH-CH-N-)
3b		6.89-7.38(m,8H,Ar-H; 8.01(d,1H,-NH-amide); 4.86(d,1H,-CH-S); 5.39(s,1H,OHphenolic); 4.64(d,1H,N-CH-CH-Cl); 5.23(d,1H,NH-CO-CH-Cl) 3.69 (s,3H,-COOCH3); 1.44(s,6H,-CH3).

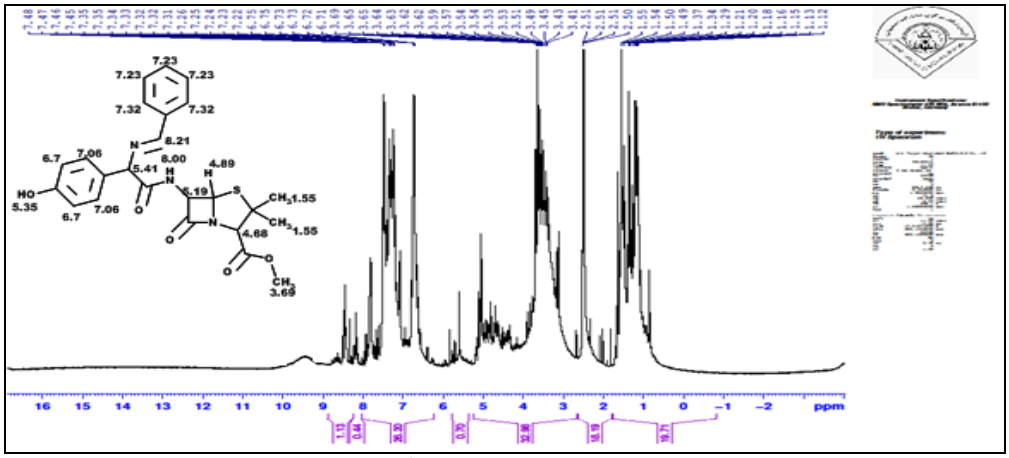


Figure-1: ^{1}HNMR for compound 2a

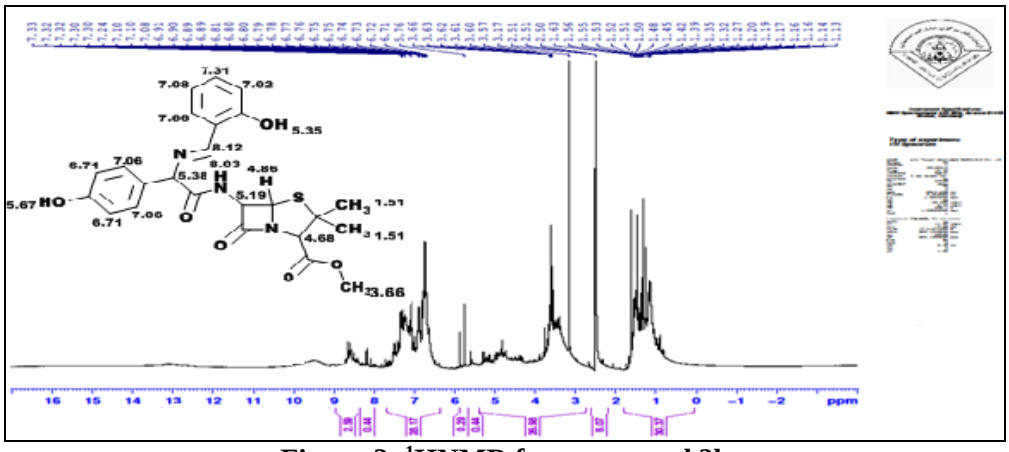


Figure-2: ^{1}HNMR for compound 2b

Date of acceptance: 25-1-2015

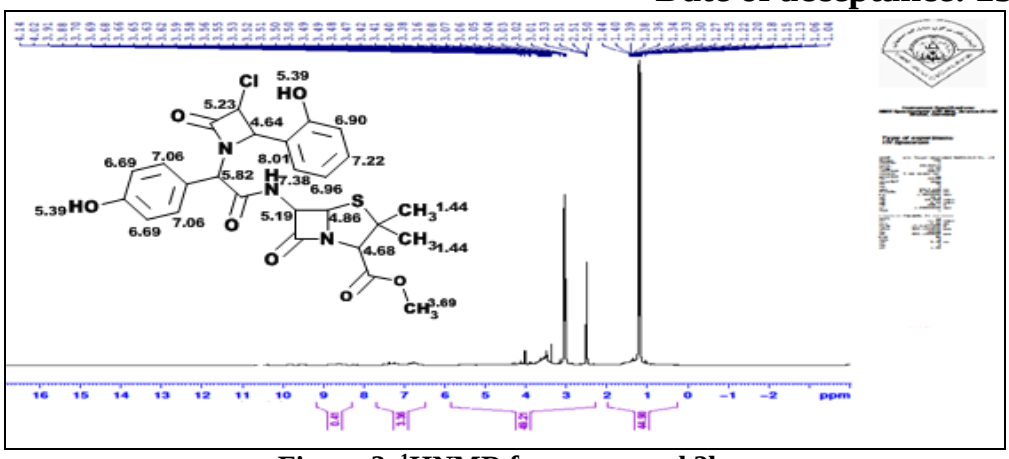


Figure-3: ^{1}HNMR for compound 3b.

Antibacterial activity:

The newly synthesized compounds 2-azetidinones 3(a-j) were tested for antibacterial activity against some bacterial species positive gram bacterial (*Staphylococcus aureus* and *Bacillies*) and negative gram bacterial (*Escherichia coli* and *Pseudomonas aeruginosa*) using DMSO as solvent to get desired concentration (400µg/ml) and the standard was amoxicillin.

The zone inhibition was measured in mm using agar well- diffusion method[14] . The results of activity listed in table-4.From table were found the compounds 3d and 3i showed inhibition antibacterial against all bacterial species and more than standard and the compound 3g showed more inhibition antibacterial than amoxicillin.

Table-4: Antibacterial activity of synthesized compounds 3(a-j) zone inhibition in mm.

Comp. Code.	*Staphylococcus aureus*	*Bacillies subtilus*	*Escherichia coli*	*Pseudomonas aeruginosa*
3a	36	20	10	-
3b	35	21	10	-
3c	32	20	11	-
3d	35	24	13	11
3e	34	25	11	-
3f	33	24	11	-
3g	33	30	13	-
3h	37	24	11	-
3i	32	23	14	11
3j	34	20	12	-
Amoxicillin	32	21	11	-
DMSO	-	-	-	-

[C]: 400µg/ml
Zone inhibition: (-) no inhibition zone

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Date of acceptance: 25-1-2015

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