Synthises and characterization of New fructosyl N-ampicillin derivative as possible prodrug

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ABSTRACT
"Prodrugs Approach" is a versatile approach in solving the problems associated with drug molecules. Ampicillin drug has some side effects i.e. absorption, toxicity, distribution, instability, formulation etc. These side effects can be reduced by "Prodrugs Approach". In the present research work a new carbohydrate derivative of N-ampicillin was prepared by reacting the appropriate 1-chloro-diacetone fructose with protected ampicillin anhydrous to obtain a new ampicillin amino derivative as possible prodrug , that has more water–solubility than parent drug make to reduce the dose given to patient and reduced the dugs side effect . The synthesized compounds were identified using U.V, FT-IR and 1H-NMR spectra and it was equal to expected. The synthesized compound was tested for their antifungal and antibacterial activities in vitro. However, detailed kinetic studies of chemical and potential enzyme hydrolysis still remain to be done.

تحضير وتشخيص مشتق فركتوسيل N- امبسلين الجديد كملازم دواء محتمل

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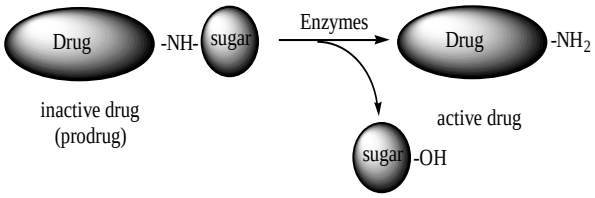
قسم الكيمياء/ كلية التربية للعلوم الصرفة /جامعة كربلاء
الكلمات المفتاحية : امبسلين ، ملازمات الدواء

الخلاصة
ملازمات الدواء تم ابتكارها للتقليل من الاثار الجانبية للادوية المتعلقة بالامتصاصية والسمية والتوزيع والثباتية وغيرها والتي يمكن التقليل منها باستخدام " ملازمات الدواء " يتضمن البحث تحضير مشتق أميني جديدة لدواء الامبسلين يكون فيها جذر ألامين سكر الفركتوز وذلك بتفاعل المركب 1 – كلورو ثنائي اسيتون فركتوز مع مجموعة ألامين للامبسلين المحمي للحصول على ملازم دواء محتمل للامبسلين وذلك لزيادة الذوبانية و الامتصاصية له مما يمكن من تقليل كمية الدواء المعطى للمريض والتقليل من اعراضه الجانبية. تم تشخيص المركبات المحضرة بواسطة طيف الأشعة تحت الحمراء والأشعة فوق البنفسجية وكذلك مطيافية الرنين النووي المغناطيسي وكانت النتائج مطابقة لما هو متوقع . الدراسات الحركية والاختبارات البايولوجية لمشتق الامبسلين الجديد هي قيد الانجاز.

1. INTRODUCTION

Ampicillin (1) is the second most used penicillin in medical practice of antibiotics since it is active versus Gram-positive and Gram negative bacteria[1-3] , acid resistant because of NH_2 group[4], non-toxic and sensitive to penicillinase, also causes diarrhea because of the poor absorption through the gut wall. This problem comes from the dipolar nature of ampicillin molecule since it has both a free amino group and a free carboxylic acid function [5]. This problem can be alleviated by using a prodrug in which one of the polar groups is masked with a protecting group. This group is removed metabolically once the prodrug has been absorbed through the gut wall.[6,7]



A considerable number of ampicillin prodrugs have been developed by converting of carboxylic acid to their ester derivatives to increase water-solubility,chemical stability, improved pharmacokinetics and reduce side effects[8-11]. Amino derivatives of ampicillin had proved to be useful prodrugs[12-16]. Carbohydrate drug conjugates connected by potentially metabolisable sacrificial linkages have high potential utility as prodrugs in which glycan moiety affords both protection and specific transport properties.[17] It is known that some carbohydrate-drug derivatives have the activity[18]. When a drug is orally administered, solubilization in the drug is essential for bioavailability because only the dissolved drug can be absorbed[19] therefore, the solubility of a drug directly affects its clinical application[20]. Ampicillin solubility increases with the pH. This behavior can be explained by its determined values of the acid group pK (2.66) and amine group pK (7.24) and calculated of its isoelectric point (4.95). Hence, above the pH correspondent to its isoelectric point the number of ampicillin molecules with a neutral charge (which is the most insoluble form) decreases leading to higher solubility values. This effect becomes more important for pH above 7.0. In the light of these results, the present work is aimed to develop carbamate-linked sugars (fructose) because this linkage is stable in aqueous solution, and could be hydrolyzed by glycosidases[21] and no other toxic groups are released from the linkage portion of the molecule. Also the solubility of the new derivative was expected to be more than free ampicillin. All synthesized compounds have been characterized on the basis of their m.p, U.V, FTIR and ^{1}H-NMR.

2. MATERIAL AND METHODS

Materials:

For anhydrous reaction, glass ware was dried over night in an oven at 120^{0}C and kept in a desicator over anhydrous $CaSO_4$ or silicagel. Reagents were purchased from Fluca Sigma (st. Louis.USA) .Solvents including dichloromethan, chloroform, carbon tetrchloride and hexanes were distilled over CaH_2 under nitrogen. Absolute methanol and ethanol were purchased from Merck (Germany) and used as received. Ampicillin anhydrous was supplied from Samarra drug industries. Samarra ,Iraq . The purity of this compound is checked according to m.p, and Meric index. Melting points were recorded using Gallinkamp electro thermal apparatus and were uncorrected. (TLC) was performed on glass plates coated with 0.25mm layer of silica gel to follow chemical reactions. Purity of the prepared compounds was checked by TLC – plated, 20x20 cm of silica gel 60 F250 with 0.25 mm thickness, Merck, Germany. Chromatograms were eluted by Aceticacid: Water:n-butanol. The chromatographic spots were detected by a reaction with iodine vapor, or 2.5% phospho-molybic acid in ethanol with heating. Purification on Silicagel refers to gravity column chromatography on Merck Silicagel 60 (particale size 230-400 mesh). Infrared spectra (IR) were recorded using FTIR-8400S- shimadzu spectrophotometer using KBr disk. Ultraviolet–Visible spectra were recorded using 402 U.V spectrophotometer, and ethanol water solution

was used as a solvent. H-NMR spectra were recorded on a Brucker 400 MHz spectrometer in $CDCl_3$.

General methods for the compounds synthesis

Syntheses of 2,3:4,5-di-O-isopropyl- idine-β-D-fructopyranos (8)

Compound (8) was prepared according to previously published procedure.[20]

***Syntheses of* 1-*chloro-2,3:4,5-di-O-isoprop- ylidine-β-D-fructopyranose* (9)**

Compound (8) was refluxed with anhydrous carbon tetrachloride (CCl_4) in the presence of triphnyl phosphen (Ph_3P) at 70^0C for 90 hr.s. triphnyl phosphen oxide was separated from the mixture after 10 hrs the cooled solution was filtered through kieselguhr evaporation under reduced pressure and purification of the residue by using of column chromate-graphy (EtOAc) afforded (9). FTIR (KBr cm^{-1}): (2983), (C-H str), (1250-1050 C-O-C str) ,(650 C-Cl):H-NMR (δ 1.55ppm,H aliph.) ,UV:(λ_{max}) 249 .The H-NMR spectra is shown in fig.1

The ***Synthises of N-(diacetone fructose) ampicillin* (10).**

carboxylic acid of ampicillin (1) was protected with $(C_2H_5)_3SiCl$ to triethylsilyl ester, then stirring with 1-chloro-2,3:4,5-di-O-isopropylidine fructopyranose (9) in the presence of Et3N in CH3CN for 5.hrs. at 25^0C to produce ampicillin derivative (10) in 35 % yield . FTIR (KBr cm^{-1}): 3305 (N-H), 3056 (C-H arm. str), 2975 (C-H alp. str), 1774 (C=O. str) 1650(C=O str. Amide) 1650(C=O str. lactam ring). ^{1}H-NMR : (δ ppm) 1.35 (H aliphatic), 7-7.6 (H aromatic) . UV: (λ_{max}) 234 and 321. The H-NMR spectra is shown in fig.2

***Synthises of N-(fructose) ampicillin* (7)**

Compound (10) was dissolved in (10 ml) of chloroform then stired with 0.5 N (HCl) for 10 hrs. at 25 C^o to produce N-(fructose) ampicillin (7). FTIR (KBr cm^{-1}): 3380 (O-H str), 3320 (N-H str), 1670 (C=Ostr amide),1692(C=O str. lactam ring).H-NMR:(δ ppm)1.35(H aliphatic), 7-8.1 (H aromatic) . UV: (λ_{max}) 230 and 325. The H-NMR and FT-IR spectra are shown in fig. (3,4) .

3. RESULTS AND DISCUSSIONS

Some of ampicillin amino derivative prodrugs have antibiotic activity. In the light of these results, the research was aimed to synthesized carbohydrate compound that has an ampicillin side chain to evaluate their antibiotic activities also the sugar may increase ampicillin solubility also has no any toxic effect after releasing. In the first step, acetalation of *β*–D-fructose to 2,3:4,5-di-iso- propylidene-*β*-D-fructopyranos(8) to protect the hydroxyl groups at C-2,-3,-4and-5 leaving the hydroxyl group at C-1 free, then converted to halo fructopyranose derivative (9). The carboxylic acid of ampicillin(1) was protected with $(C_2H_5)_3SiCl$ to its triethylsilyl ester could be reacted safely with a halo fructopyranose derivative. The synthesis of these compounds was carried out according to the steps outlined in scheme (1). The physical properties and spectroscopic data are given in table (1) (2). The following compounds were synthesized: (7),(8), (9) and (10). All compounds except (8) are new compounds. Compound (8) was previously synthesized,[20]. Therefore, its spectroscopic

data are reported here, together with the data for the new compounds. The purity of the synthesized compounds was checked by performing TLC. The structures of the synthesized compounds were determined on the basis of their FTIR ,U.V and ^{1}H-NMR.The physical properties and the spectroscopic data are given in table (1) and (2).

FTIR studies.- In the FTIR spectra of derivatives (9) showed disappearance of ν (O-H) absorption bands and appeared bands at 650 cm^{-1} for C-Cl group .Compound (10) showed disappearance of ν (N-H) and ν (O-H) of carboxylic acid absorption bands and showed absorption bands at 1774 cm^{-1} for C=O of ester group .The deprotection of compound (10) show broad band at 3380 cm^{-1} for O-H group as shown in fig. (4). This and other IR absorptions are given in table (2).

UV–Vis studies.- UV–Vis spectra of the compounds were measured in DMF using 10^{-2} and 10^{-4}M, for compound 8 and 9 showed very strong bands at (291 nm) and (249 nm).This absorption due to n→δ* transition, for ampicillin derivatives (10 and 7) showed two very strong bands at (234 nm, 321) and (230 nm,325 nm) this absorption due to n→π* and π →π* transition as seen in table (2) . *H-NMR studies*.- ^{1}H-NMR spectra of compound (9) showed signals at δ= (1.55) ppm belong to aliphatic, compound (10) show different types of signals between δ=(0.7) ppm to δ=(1.55) ppm for aliphatic, protons at δ=(7.01-7.11) ppm for aromatic, compound (7) show the disappeared signals of protected group protons, and showed signal atδ=(10.5) ppm for carboxylic acid as seen in fig.(3). *Solubility studies*.- the solubility study of the ampicillin derivative (7) was determined as Gude et al. [23] using different types of solvents improve the increasing solubility than free ampicillin.

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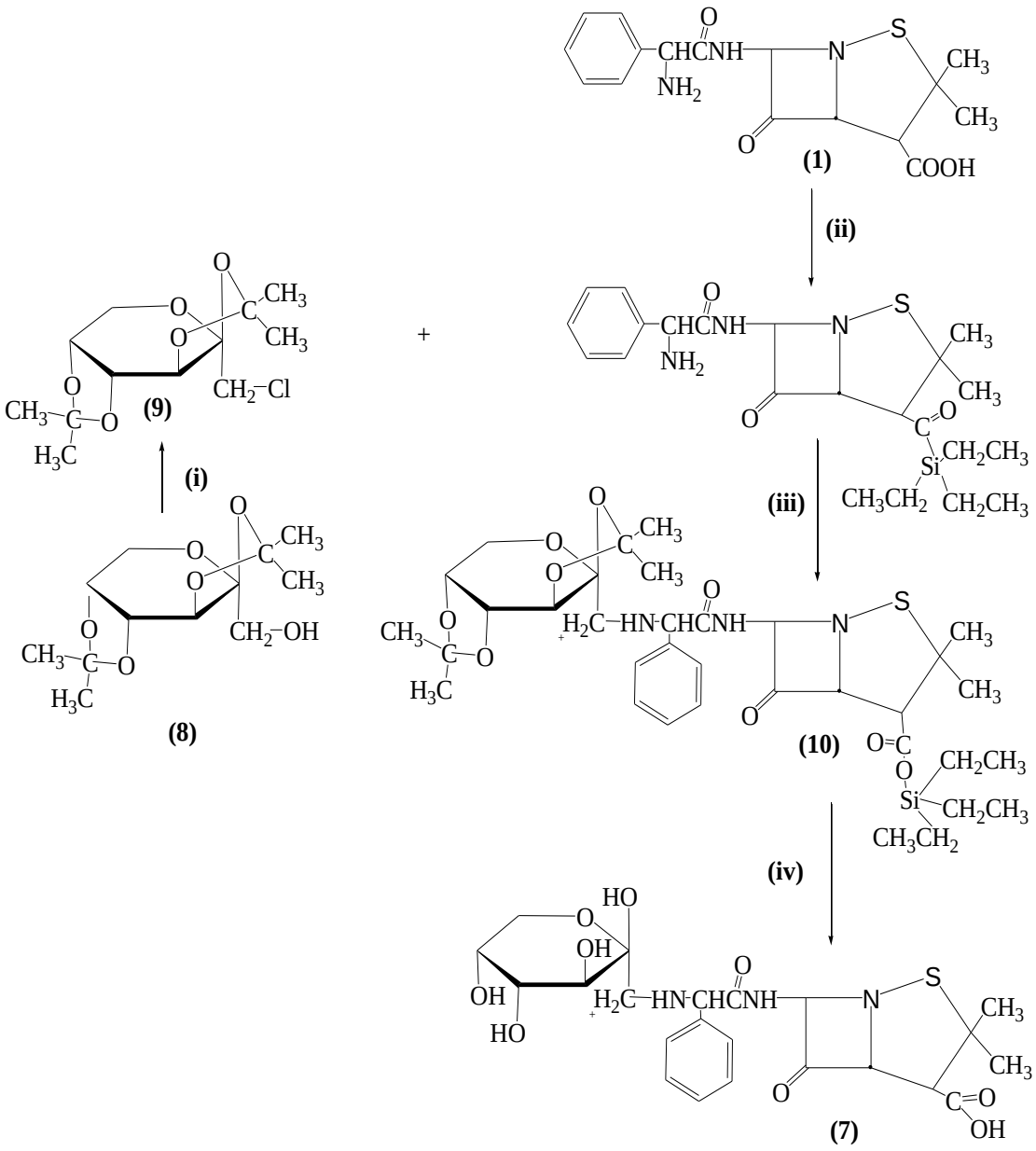

***Scheme 1*. Synthesis of compound (7,8,9,10)**

i) $(Ph)_3P$,CCl_4 , 90 hr, 70^0C ii) $(C_2H_5)_3SiCl$, 3 hrs., iii) CH_3CN, Et_3N 5.h, iv) HCl, 10 hr , $25\ ^0C$ **.**

Table(1):The Physical data of compounds (7-10).

Com. No.	Molecular formula	Color	Melting Points °C		Yield %	R_f Valuo
			Found	Reported		
8	$C_{12}H_{20}O_6$	Yellow	96	97	66	0.8
9	C12H19O5Cl	Yellow	140	-	82	0.7
10	C30H41O9N3SSi	Brown	Oil	-	35	0.6
7	C22H29O9N3S	White	95	-	50	0.65
Ampicillin	C16H19O4N3S	White	-	191	-	0.9

Table(2):The spectroscopic data of [7-10].

comp. No.	FTIR spectral data cm^{-1}			^{1}H-NMR spectral data Ppm			λ_{max} (nm)
8	**ν(OH) Alcohol**	**ν(C-H) aliph.**	**ν(C-O-C) acetyl .**	**δ(H) Arom.**	**δ(H) aliph.**		291
	3311	2983	1250	-	-	-	
9	**ν(C-H) aliph.**	**ν(C-Cl)**	**ν(C-O-C) Acetyl**	**δ(H) Arom.**	**δ(H) aliph.**	**δ(H) H-C-Cl**	249
	2983	650	1250	-	1.55	3.06	
10	**ν(N-H) Amine**	**ν(C-H) arom.**	**ν(C=O) Ester**	**δ(H) Arom.**	**δ(H) aliph.**	**δ(H) lactam**	234 321
	3305	3056	1774	7-7.6	0.9-1.35	6.09	
7	**ν(O-H)**	**ν(N-H) amine**	**ν(C=O) Acid**	**δ(H) arom.**	**δ(H) aliph.**	**δ(H) Acid**	230 325
	3380	3320	1692	7-8.1	1.35	10.5	

Table(3):The solubility of compound [7] compare to ampicillin .

comp. No.	Water	Ethanol	Acetone	Chloroform	Ether	DMF
7	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
Ampicillin	insoluble	insoluble	insoluble	Insoluble	Insoluble	Soluble

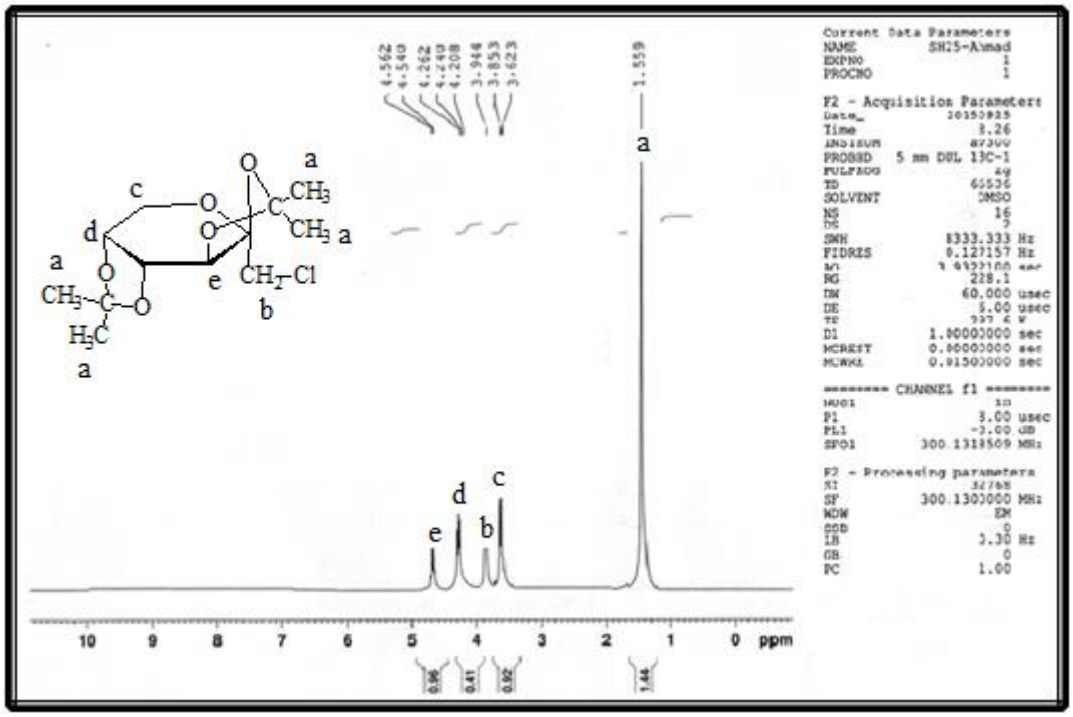

Fig. (1) H-NMR spectra of compound (9)

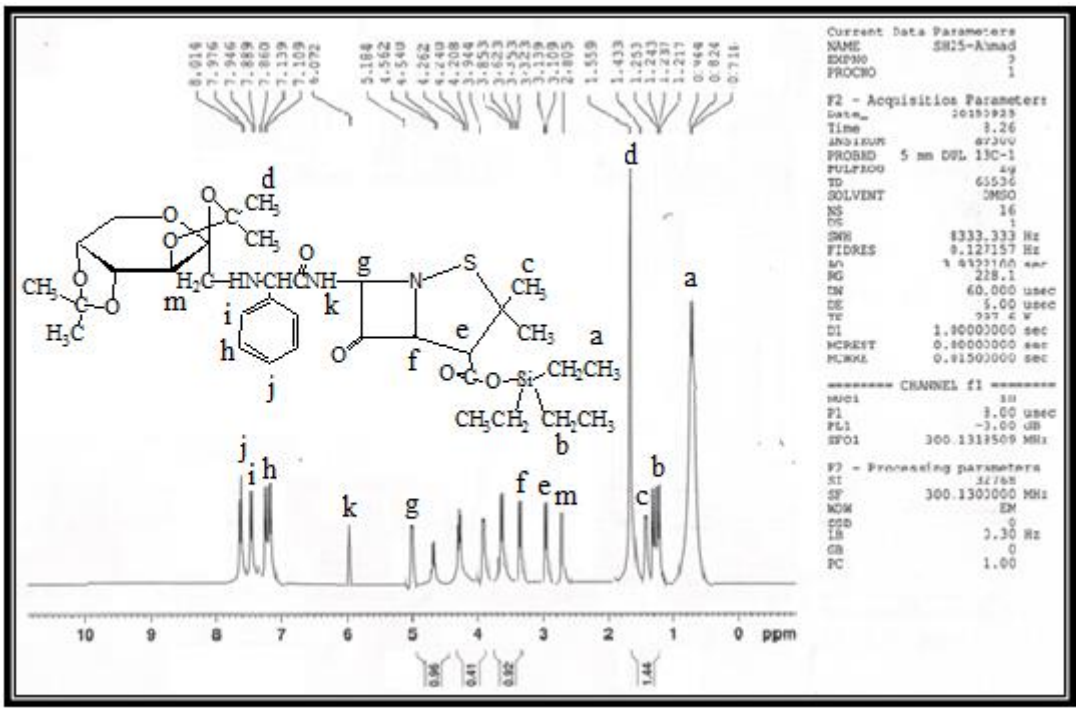

Fig. (2) H-NMR spectra of compound (10)

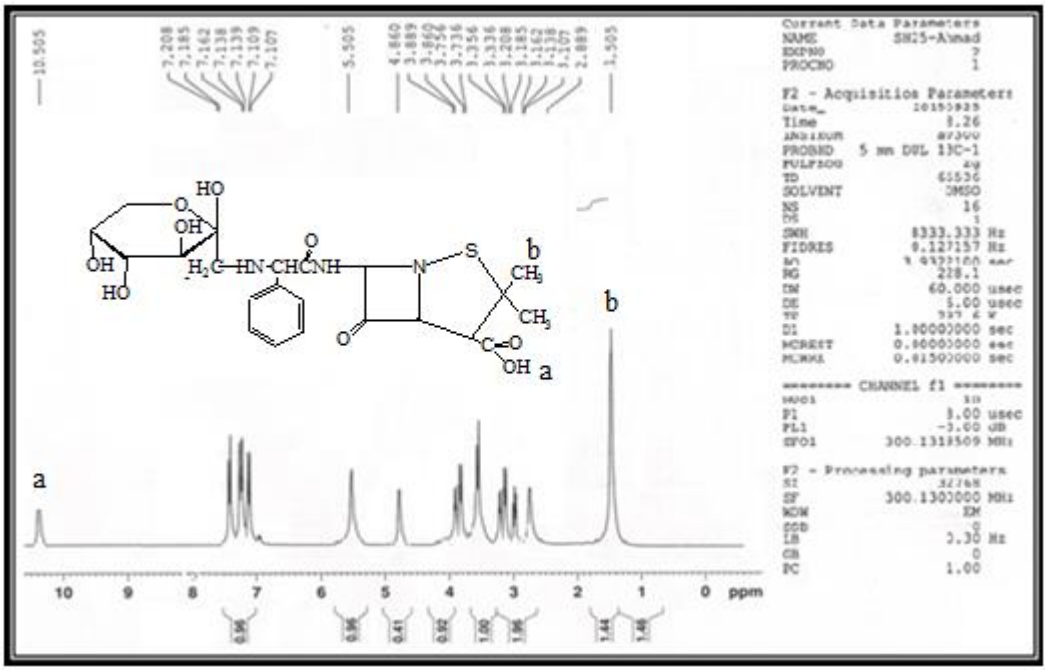

Fig. (3) H-NMR spectra of compound (7)

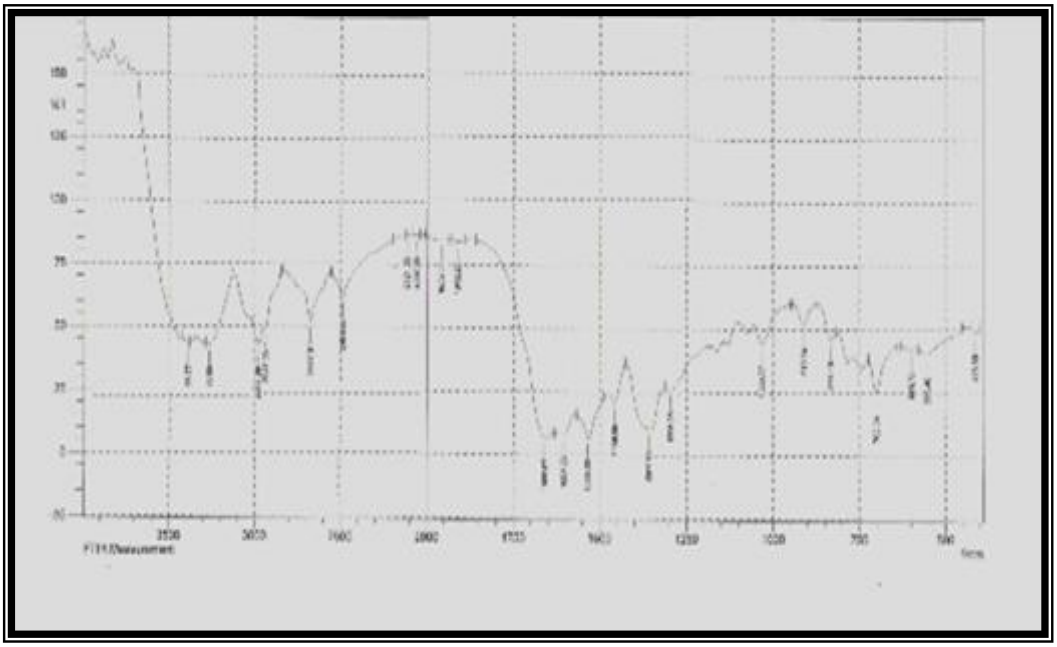

Fig. (4) FT-IR spectra of compound (7)

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