

Synthesis of New Derivatives of β - Lactam Antibiotics

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Abstract: New Derivatives for known β -lactam antibiotics were synthesized utilizing the free amino group present in the parent compound as the site for derivatization. The objectives for this study are to have new compounds which could have an increases potential resistance to the degradative enzymes knowing to be able to destroy the antibacterial activity of β -lactam antibiotics– Besides, these new derivatives could be congeners of known agents, or may be a potential pro-drugs for these agents.

Key words: β -lactam , 3-nitro phthalic anhydride , antibiotics.

Introduction:

β -Lactam antibiotics are of considerable value in the chemotherapy of bacterial infections because of their great potency, wide spectrum of activity, and low incidence of adverse reactions⁽¹⁾. These drugs, however, with bacterial may be viewed as protagonists in a never ending battle. The antimicrobial activity of these antibiotics are limited by the growing resistance of the pathogenic bacteria^(2,3).

Actually various mechanisms of bacterial resistance to these agents are operative. In case of penicillins, the microorganism may be intrinsically resistance because of structural differences in the penicillin binding proteins (PBPs) that are the targets of these drugs⁽⁴⁾.

Other instances of bacterial resistance to the β -lactam antibiotics are caused by the inability of the agent to penetrate to its site of action⁽⁵⁾. The most important challenge to these β -lactam antibiotics coming from resistance strains of bacteria that can destroy them enzymatically⁽⁶⁾. β -lactamases, however, are the most important enzymes in this situation.

Different microorganisms elaborate a number of distinct β -lactamases in which the substrate specificities of some of these enzymes are relatively narrow, and these often are described as either penicillinases or cephalosporinases⁽⁷⁾.

Other broad- spectrum enzymes are less discriminant and can hydrolyse a variety of β -lactam antibiotics⁽⁸⁾.

In this communication we wish to report the synthesis of new derivatives for two currently used β -lactam antibiotics, ampicillin and cephalexin. These two drugs have free amino in their side chain (scheme 1,2). This amino group was allowed to react with phthalic anhydride in two different reaction conditions to produce new two derivatives for each parent drug (Compounds 3 – 6, scheme 1-4).

These derivatives were designed to be either new analogs or may expected to be prodrugs⁽⁹⁾.

Experimental Section:

Material and Methods:

Material:

Ampicillin trihydrate and cephalexin anhydrous were supplied from Samarra Drug Industries, Samarra, Iraq. The purity and identity of these compounds were checked according to B.P and Pharmaceutical Codex. Triethylamine (TEA) and 3-nitro Phthalic anhydride were purchased from Hopkins and Williams, LTD, England. The remaining chemicals were of reagent grade, and were used as such without further purification, since they are of the highest commercially available purity.

In this work, the following microorganisms were used in determining the antimicrobial activities of the prepared compounds.

Salmonella typhi ,Escherichia Coli,Staphylo coccus aureus,
Candida albicans ,Bacillus ceruse.

Melting points were recorded using Gallinkamp electro thermal apparatus and were uncorrected. Thin – layer chromatography (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 the following solvent systems:

- A) Acetic acid: Water: n- Butanol (1:1:2)
- B) Acetone: Benzene: n- Butanol (1:3:1)
- C) Chloroform: Ethanol (3:1)

The chromatographic spots were detected by reaction with iodine vapor, or by observing them under ultraviolet light.

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. Elemental microanalysis were performed at the University of Damascus, college of science, using CHN analyzer, type Elementer- vario Micro CUBE.

Synthesis Procedures:

Synthesis of (7R) -3- Methyl-7- [α -D- (3-nitrothaimido)- Phenylglyclamino -3-Cephem-4-Carboxylic acid (Compound.3).

This compound was synthesized by a method of Fling *etal*⁽¹⁰⁾, however some modifications to the procedure were found quite necessary.

In this procedure equimolar amounts (0.1 mole) of cephalexin anhydrous and 3-nitro phthalic anhydride were fused together in a pyrex round bottomed flask. The fusion was performed in an oil bath pre-heated to 145-150°C. The reaction mixture was kept at this temperature for 30 min. During the first 10 min. the mixture was stirred occasionally to allow proper mixing of the reactants. The sublimed 3-nitro phthalic anhydride which deposited on the walls of the flask was pushed down into the reaction mixture by means of glass rod. The mixture was left undisturbed during the next 20 min. The reaction mixture was carefully cooled until the liquid mass solidified, and was dissolved in ether and filtered. To the filtrate, n- hexane was added until turbidity persisted. The precipitate was filtered after freezing cool for 5hr- to give compound No.3. The percent yield, m.p and Rf values are given Table I, Table II shows the main IR bands of the prepared compound in term of frequency (cm⁻¹). Table III shows the CHN values,where $\lambda_{max}=296$ nm(IR.Fig 2)

Synthesis of (6R) -6- [α - D- (3-nitroPhthalimido)–Phenylglyclamino]- Penicillanic acid,(Compound 4) .

This compound had been synthesized by reaction of ampicillin trihydrate and 3-nitroththalic anhydride using the same procedure previously described for the synthesis of compound 3.

The percent yield, m.p, and chromatographic Rf values are given in table I, table II shows the values of the IR frequencies of the main functional group. Table III shows the results of CHN analysis for this compound. where $\lambda_{\max} = 250\text{nm}$.

Synthesis of (7R) -3-Methyl -7- [α -D- (2- Carboxy – 3-nitroPhenyl Carbonyl) – Phenylglycylamino] -3- Cephem- 4- Triethylammonium Carboxylate, (Compound 5).

This compound was prepared according to a method of Perron et al⁽¹¹⁾. In this modified procedure, a mixture of cephalexin anhydrous (36.5g, 0.1 mol) in 60 ml. of dimethylformamide (DMF) and TEA (42ml., 0.3mol) was stirred for 1hr. at 0 – 5 °C. To this solution there was added a solution of (14.8g, 0.1 mol) of 3-nitrophthalic anhydride in 60 ml of DMF.

The addition was dropwise at a rate which keep the temperature below 10°C. The mixture was then stirred for 5hr. at 25°C. The yellow solution was filtered and diluted with 600ml. of anhydrous ether. The oily precipitate was collected and slowly crystallized on prolonged cooling, scratching, and addition of cold ether affording compound 5, where $\lambda_{\max} = 318\text{nm}$

The percent yield, m.p, and chromatographic Rf values are given in table I. The IR spectram are given in table II. Table III shows the results of CHN analysis.

Synthesis of (6R) – [α -D- (2- Carboxy-3-nitrophenylCarbonyl) – Phenylglycylamino] -3- Triethyl ammonium Penicillanate, (Compound 6) .

This compound had been synthesized by the reaction of ampicillin trihydrate and 3-nitro phthalic anhydride, utilizing the method of Perron et al previously described.

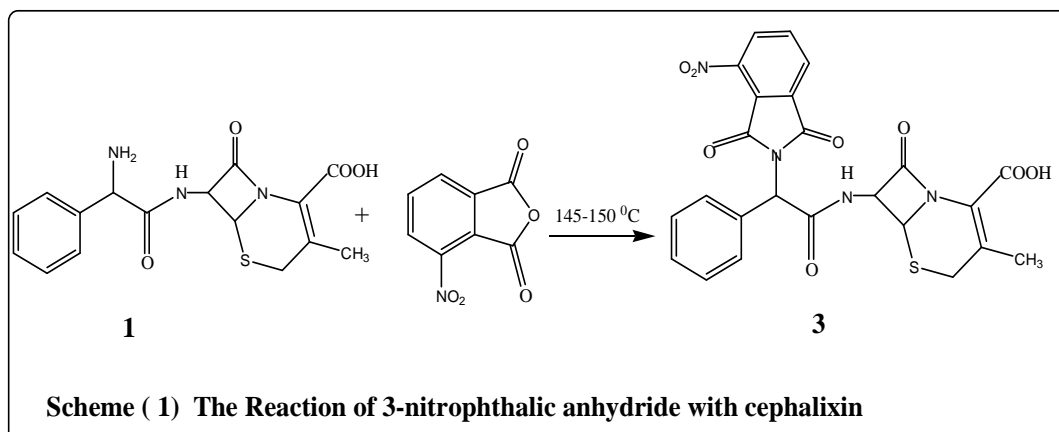
The percent yield, m.p., and Rf values are given in table I. table II shows the main IR frequencies of this compound, while table III shows the results of CHN analysis obtained, where $\lambda_{\max} = 261\text{nm}$

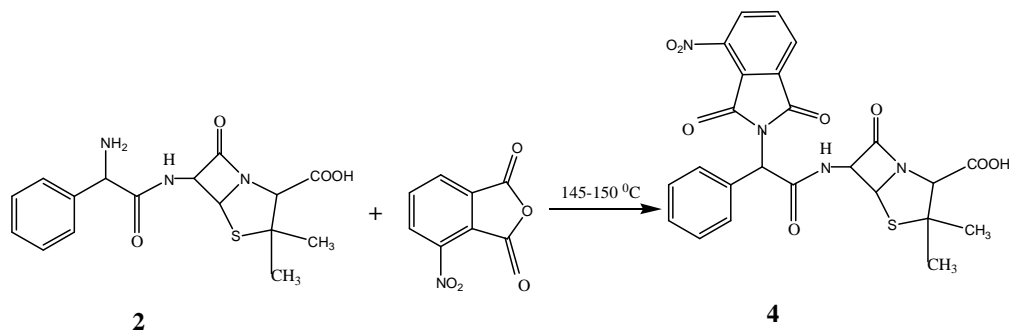
Preliminary Microbiological Assay

All procedures were conducted under sterile conditions using sterile media and glass wares. Antimicrobial activity was carried out by agar diffusion method⁽¹²⁾.

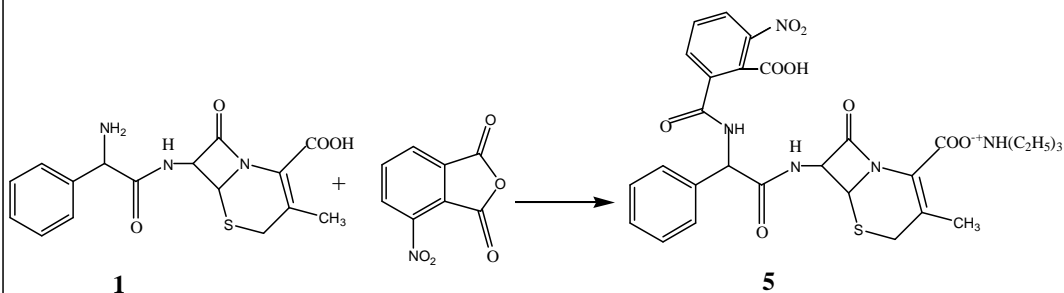
The antimicrobial activity of the four compounds was determined using different concentrations (50 microgram and 100 microgram). Ampicillin and cephalexin were used as control.

In this procedure tryptic soy agar media was poured in glass plates (20ml for each 9cm in diameter glass plate). After solidification, agar plates were seeded with a loopfull (0.01 ml) of a 24 hr. culture of bacteria. A hole of 6mm in diameter were made in the solid medium by the use of cork-borer which is conducted to vacuum pump. Then 50 microgram amount (dissolved in Ethanol of each compound to be tested was added to each hole. After 24 hr of incubation, the diameter of the inhibition zone of each compound was measured. This procedure was repeated using 100 microgram amount of each compound. Table IV shows the inhibition zones of each compound tested.

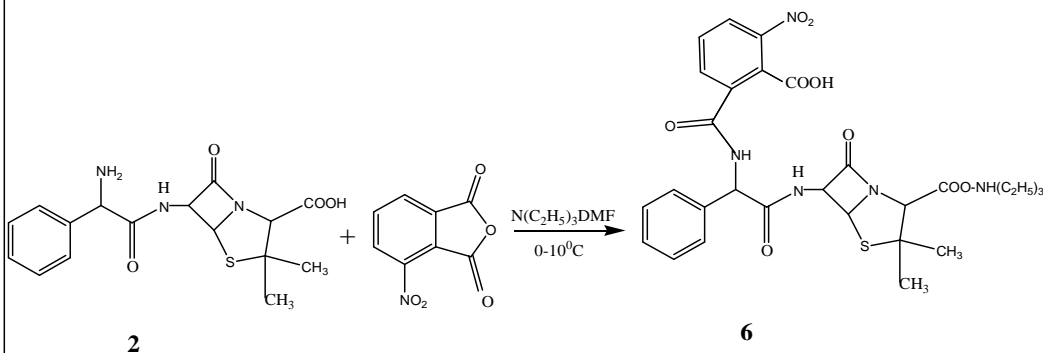




Scheme (2) The Reaction of 3-nitrophthalic anhydride with Ampicillin



Scheme (4) The Reaction of 3-nitrophthalic anhydride with cephalixin at 0-10 °C



Scheme (5) The Reaction of 3-nitrophthalic anhydride with Ampicillin at 0-10 °C

Results and Discussion

Reaction of 3-nitro Phthalic Anhydride with the Amino Group of Ampicillin and Cephalixin at Elevated Temperature.

3-nitroPhthalic anhydride is one of the early agents used for the protection of the amino function of amino acids during peptide synthesis⁽¹³⁾. The reaction of phthalic anhydride with the desired amino acid is performed by either fusion at a temperature near 180°C, or by allowing it to be heated under reflux with the

amino acid in dioxane or toluene suspension⁽¹⁴⁾. The fusion method applied in this work was found to be a simple one, less time consuming and gave products with high yield.

The suspension method was found, because of prolong heating, to caused partial decomposition of cephalixin and ampicillin. To avoid high temperature in our procedure, the fusion was performed at relatively lower temperature (145-150°C) with increasing the time course to 30 min.

In this method, 3-nitro phthalic anhydride was condensed with the amino function of ampicillin (for example) according to the general equation illustrated in scheme -2-. The proposed mechanism for this reaction had been involves nucleophilic attack of the amino group on one of the carbonyl carbon atoms of 3-nitrophthalic anhydride .

Reaction of 3-nitro Anhydride with the Amino Group or Ampicillin and Cephalixin at Low Temperature (0-10° C)

In this reaction we planned to furnish another carboxyl group and to form further peptide bond in the synthesized molecule. To achieve this goal, 3-nitrophthalic anhydride was allowed to react with the free amino group of cephalixin and ampicillin at low temperature in a base – catalyzed condensation reaction using TEA as a base and DMF as a solvent (scheme -4-).

The reaction performed at low temperature was found to be stopped at the step of amide bond formation. The products thus formed have been isolated as crystalline triethyl ammonium salts. It is not worthy that these products (Compounds 5 and 6) although possessing two carboxyl groups, crystallized as monotriethyl ammonium salts even when the reaction was carried in the presence of large excess of the organic base⁽¹¹⁾.

Decomposition study:

A study was conducted to measure the speed decomposition of derivatives prepared using PH 1.5 and PH7.4 , where first solution was prepared of 0.1N(HCL) ,the second solution (7.4) of phosphate buffer t he results of the speed of decomposition of compounds prepared was recorded in table(IV ,V,VI .VII) ,the time interval between each read about half an hour ± two minutes.

Antimicrobial Activity:

Preliminerry antimicrobial investigations had been carried out using standard procedures, to examine the antibacteria activity of compounds 3-6.

In this investigation the following bacterial species including Staphaureus, E.coli, and Psed. Aeruginosa, Bacillas ceruse and candida albicans and salmonella typhi were used to examine the antimicrobial activity of these compounds. As shown in table –VIII– Compound 3 showed activity to Ampicillin at 50 μ g / ml concentration in both candida and E. coli tests. Compound 4 showed activity comparable to cephalixin in Bacillus, candida and Salmonella. When these compound were examed using 100 mg/ml concentration, compounds 3, 4,5 shows activity to Ampicillin and Cephalixin. Obviously no compound in this investigation showed superior activity in comparison with the compound from which it was derived. Further studies are planned to be carried out too.

Table I: The physical data of Compounds (3-6)

Compound	Melting point C		yield%	Rf value		
	Found	Reported		S1	S2	S3
3	143-146		53	0.71	0.75	0.25
4	200-201		60	0.7	0.73	0.16
5	74-77		35	0.8	0.78	0.32
6	88-90		42	0.75	0.65	0.25
Ampicillin	----	191-192	---	0.9	0.95	0.3
Cephalixin	----	185-187	---	0.85	0.9	0.31
3-nitrophthalic anhydride	----	163-164	---	0.8	0.8	0.82

Table II: C-H-N Analysis of Compounds (3-6)

No.	Chemical formula	C-H-N					
		Found %			Calculated %		
		C	H	N	C	H	N
3	C ₂₄ H ₁₈ O ₈ N ₄ S	54.89	3.3	10.05	55.17	3.44	10.7
4	C ₂₄ H ₂₀ O ₈ N ₄ S	54.57	3.12	9.93	54.96	3.81	10.6
5	C ₃₀ H ₃₅ O ₉ N ₄ S	57.31	5.34	8.81	57.41	5.5	8.93
6	C ₃₀ H ₃₇ O ₉ N ₅ S	55.04	5.31	10.02	55.98	5.75	10.88

Table III: IR data of Compounds (3-6) Ampicillim and Cephalexim

Compound	C=O of β - lactam	C=O of carboxylic	C=O of amide	N-H bend of amide	N-H of amine	NO ₂	λ_{\max}
3	1770	1750	1650	1515	-----	Asy(1550-1475)cm ⁻¹ sy(1360-1290) cm ⁻¹ .	296
4	1780	1755	1645	1520	----- -	=	250
5	1775	1750	1650	1520	-----	=	248
6	1770	1750	1650	1515	-----	=	260
Ampicillin	1780	1750	1650	1520	3400- 3500		254
Cephalexin	1780	1755	1645	1520	3410		254

Table IV: Absorbance value of compound -3- at PH=1.5 and PH=7.4 at λ_{\max} 296nm

A at PH 1.5	A at PH 7.4
0.2264	0.2276
0.2401	0.2750
0.2278	0.2463
0.1627	0.1794
0.2350	0.3025

Table V: Absorbance value of compound -5- at PH=1.5 and PH=7.4 at λ_{\max} 318nm

A at PH 1.5	A at PH 7.4
0.1195	0.2430
0.1459	0.2696
0.1315	0.2515
0.1501	0.2933
0.1300	0.2424

Table VI : Absorbance value of compound -4- at PH=1.5 and PH=7.4 at λ_{\max} 250nm

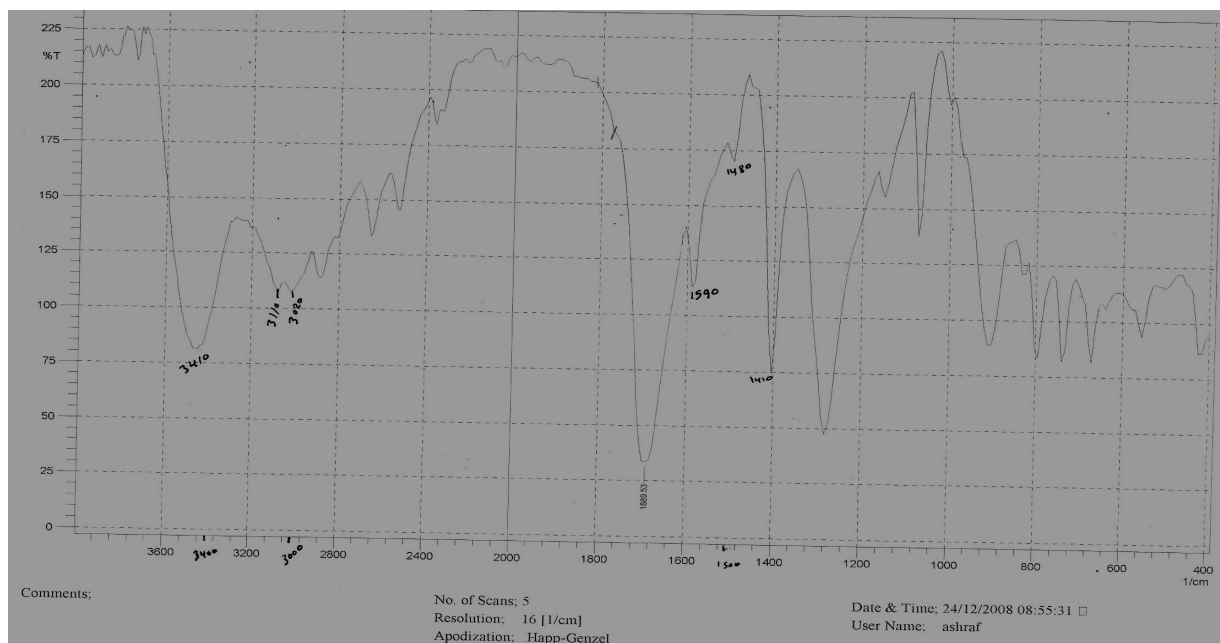
A at PH 1.5	A at PH 7.4
4.9159	4.7219
5.1113	4.9243
5.0033	4.8682
4.8980	4.9708
4.9296	4.8738

Table VII: Absorbance value of compound -6- at PH=1.5 and PH=7.4 at λ_{\max} 261nm

A at PH 1.5	A at PH 7.4
0.2603	0.2700
0.2596	0.3033
0.2285	0.2239
0.2634	0.2639
0.2453	0.2511

Table VIII: Antimicrobial activity of compounds (3-6) (Zone of inhibition in mm)

Compounds	Antimicrobial activity				
	Staph aureus	E-coil	Salmonella	Condida	Bacillus Ceruse
50 μ g/ml					
3	---	---	5	10	5
4	---	11	---	10	---
5	---	5	---	16	6
6	---	8	---	12	---
Ampicillin	30	20	20	20	15
Cephalexin	22	20	15	20	22
100 μ g/ml					
3	---	---	7	11	8
4	---	12	---	12	---
5	---	6	---	17	9
6	---	9	---	15	---
Ampicillin	31	25	25	25	17
Cephalexin	25	22	20	22	23

**Fig. 1 :** IR spectrum of pure cephalexin compound- No-1

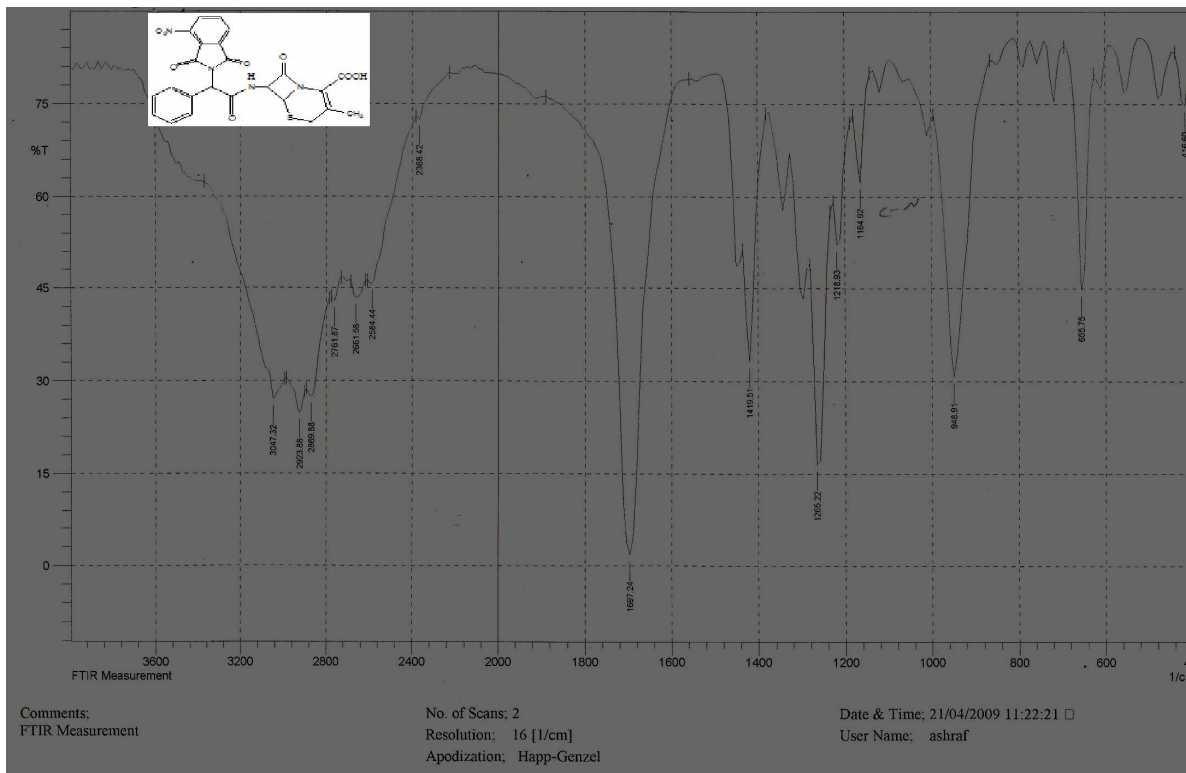


Fig. 2 : IR spectrum of cephalixin derivative- No-3

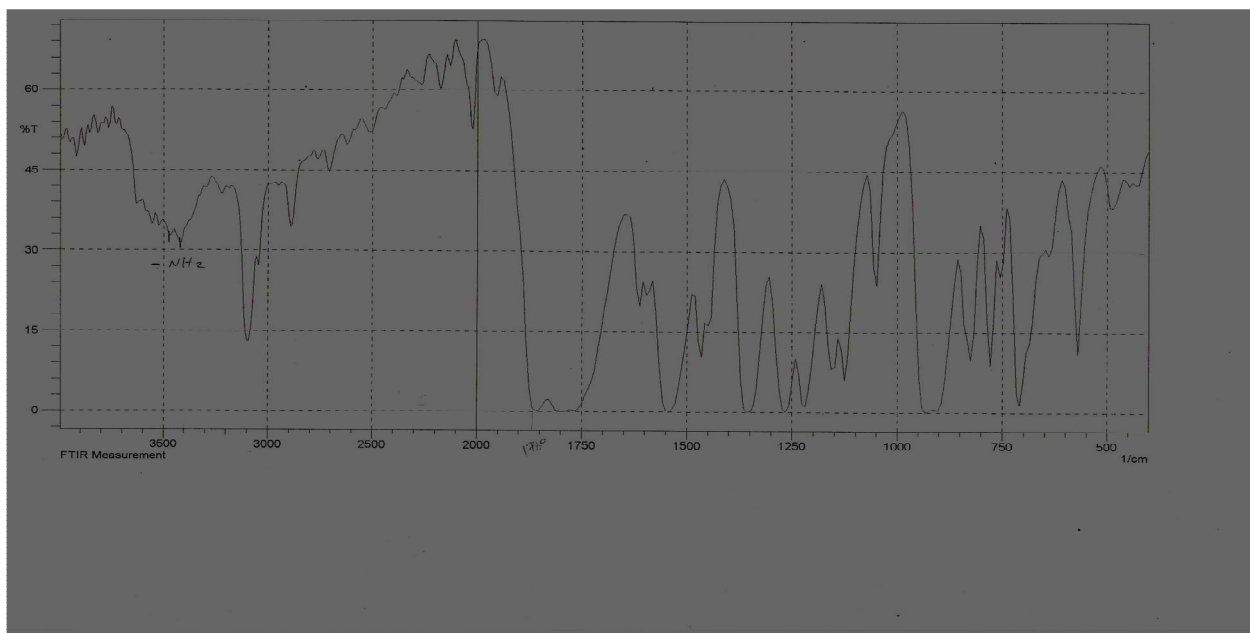


Fig. 3 : IR spectrum of pure ampicillin compound- No-2

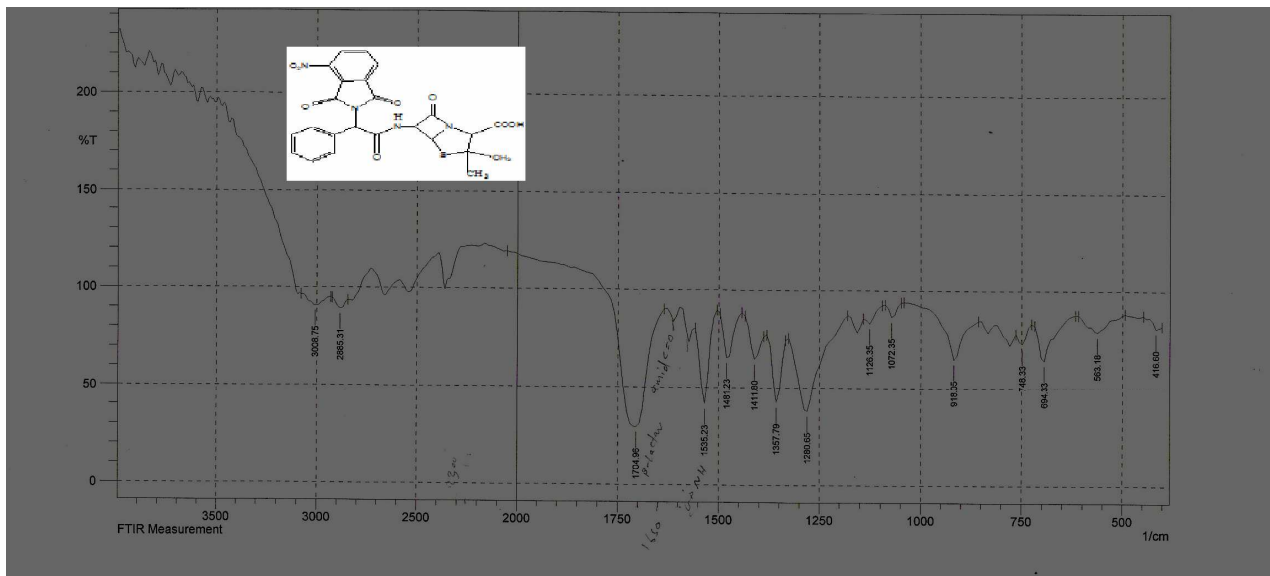


Fig. 4 : IR spectrum of ampicillin derivative- No-4

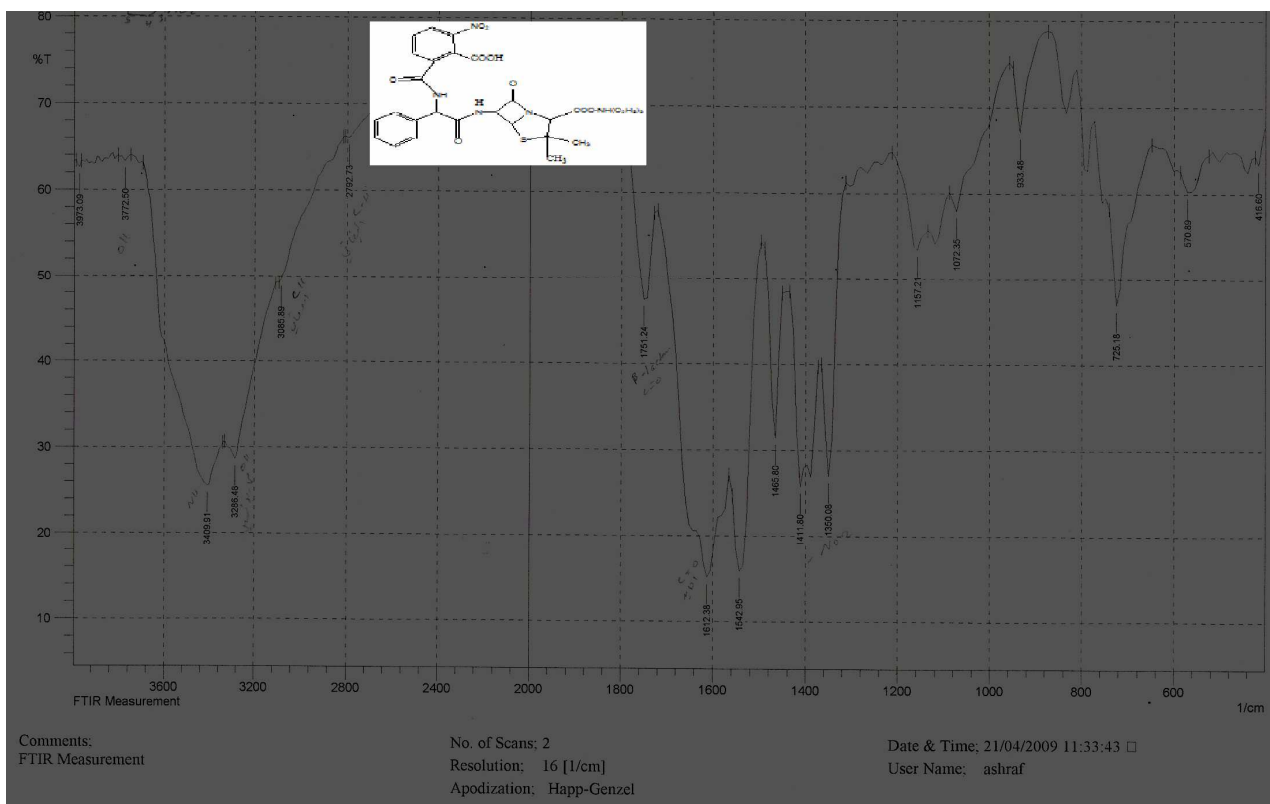


Fig. 5 : IR spectrum of cephalixin derivative- No-5

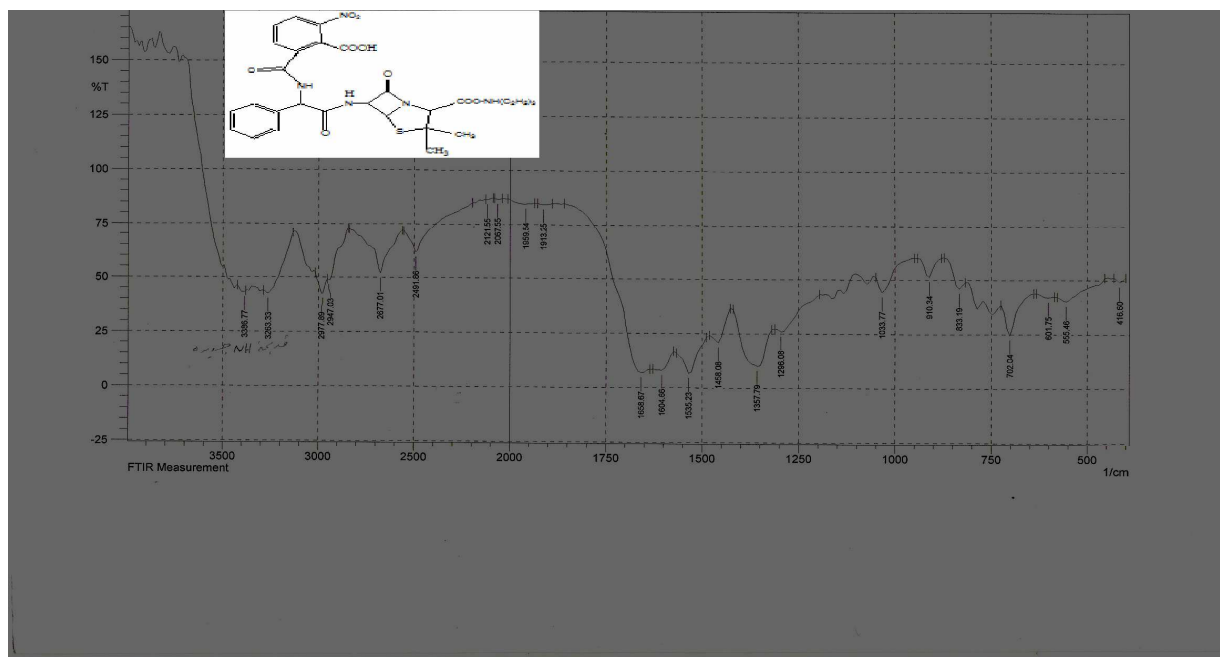


Fig. 6 : IR spectrum of ampicillin derivative- No-6

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