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 compounds as the site for derivatization. The objectives for this study are to have new compounds which could have an increased 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.

Introduction

B-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 bacteria 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 (PBP s) 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 cephalosporinasies (7).

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

In this communication , the synthesis of new derivatives for two currently used β-lactam antibiotics, ampicillin and cephalexin are described. These two drugs have free amino groups in their side chain (Figure 1). 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, Figure 2).

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 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 antimicrobial activity was determined in the university of Basrha , college of pharmacy :

1. Pseudomonas aeruginosa.
2. Escherichia Coli.
3. Staphyllo coccus aureus.
4. Candida albicans.

Melting points were recorded using Thomas Hoover electro thermal apparatus and were uncorrected. Thin- layer chromatography (TLC) was performed on glass plates coated with 0.25 mm 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 Perkin - Elmer 1310 spectrophotometer using KBr disk. Ultraviolet – Visible spectra were recorded using 402 U.V spectrophotometer, and sodium bicarbonate (1%) aqueous solution was used as a solvent. Elemental microanalysis were performed at the University of Mousel, College of Science, using CHN analyzer, type 1106 Carlo Erba.

Synthesis Procedures :

Synthesis of (7R) -3- Methyl -7- [α- D- (Phthalimido)- Phenylglycylamino] -3-Cephem- 4- Carboxylic acid (Compound no.3).

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

In this procedure equimolar amounts (0.1 mole) of cephalexin anhydrous and 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 phthalic anhydride which deposited on the walls of the flask was pushed down into the reaction mixture by means of a 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 cooling for 5hr- to give compound no.3. The percent yield, m.p and Rf values are given Table 1, Table 2 shows the main IR bands of the prepared compound in term of frequency (cm^{-1}). Table 3 shows the CHN values.

Synthesis of (6R) -6-[[α- D- (Phthalimido)- Phenylglycylamino]- Penicillanic acid, Compound 4.

This compound had been synthesized by reaction of ampicillin trihydrate and phthalic anhydride using the same procedure as previously described (10) for the synthesis of compound 3.

The percent yield, m.p, and chromatographic Rf values are given in table I, table 2 shows the values of the IR frequencies of the main functional group . table 3 shows the results of CHN analysis for this compound .

Synthesis of (7R) -3-Methyl -7- [α-D-(2′-Carboxy - Phenyl 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 of (14.8 g ,0.1 mol) of phthalic anhydride in 60 ml of DMFwas added 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, triturotion, and addition of cold ether afforing compound 5.

The percent yield, m.p, and chromatographic Rf values are given in table I. The IR spectram are given in table 2. Table 3 shows the results of CHN analysis.

Synthesis of (6R) - [α -D- (2`-Carboxyphenyl Carbonyl) - Phenylglycylamino] -3- Triethylammonium Penicillanate, Compound 6.

This compound was synthesized by the reaction of ampicillin trihydrate and phthalic anhydride, utilizing the method of Perron et al as previously described.

The percent yield, m.p., and Rf values are given in table 1. table 2shows the main IR frequencies of this compound, while table 3 shows the results of CHN analysis obtained.

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 methanol) 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.

Results and Discussion

Reaction of Phthalic Anhydride with the Amino Group of Ampicillin and Cephalexin at Elevated Temperature.

Phthalic 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 cause partial decomposition of cephalexin 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, phthalic anhydride was condensed with the amino function of ampicillin (for example) according to the general equation illustrated in scheme -3-.

Reaction of Phthalic Anhydride with the Amino Group of Ampicillin and Cephalexin 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, phthalic anhydride was allowed to react with the free amino group of cephalexin 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. (15)

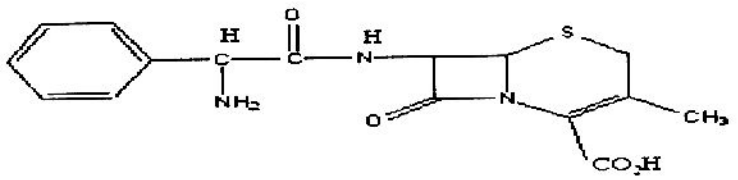
The products thus formed have been isolated as crystalline triethyl ammonium salts. It is not worthy that these products (Compounds 5 and 6) although possessing two carboxyl groups, crystallized as monotriethyl ammonium salts even when the reaction was carried in the presence of large excess of the organic base (11).

Antimicrobial Activity

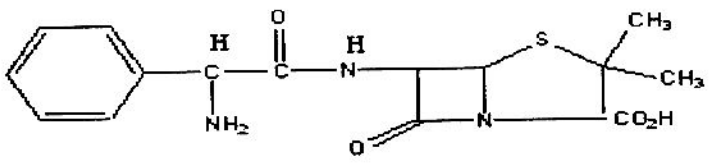
Preliminerry antimicrobial investigations had been carried out using standard procedures , to examine the antibacteria activity of Compounds 3-6 .

In this investigation the following bacterial speciesincluding Staph-aureus , E.coli , and Psed. Aeruginosa were used to examine the antimicrobial activity of these compounds . As shown in table -4- Compound 3 and 5 showed activity superior to Ampicillin at 50μg / ml coocertration in both Staph .aureus and E.coli tests . Compound 5 showed activity comparable to cephalexin in Staph-aureus test. When these compound were examed using 100mg/ml concentration , Compound 5 shows activity superior to Ampicillin. Obviously no compound in this investigation showed superior activity in comparison with the compound from which it was derived. Further studies are plained to be carried out to examined the utility of the prepared compound (3-6) as possible prodrug candidates .

Scheme (1)- The Structural Formula of Cephalexin (1) and Ampicillin (2)

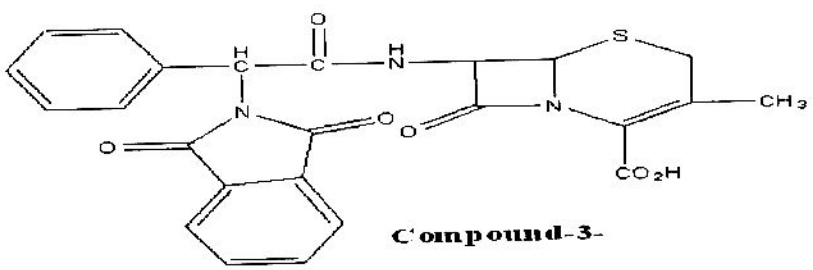


Cephalixin (1)

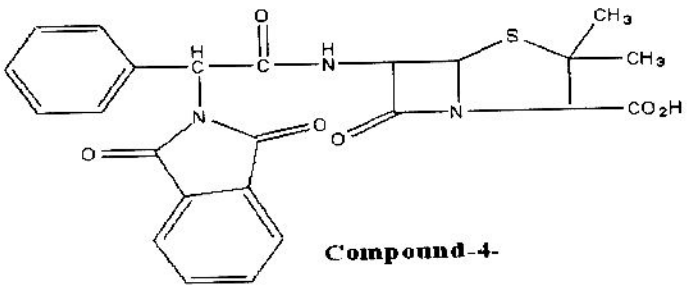


Ampicillin (2)

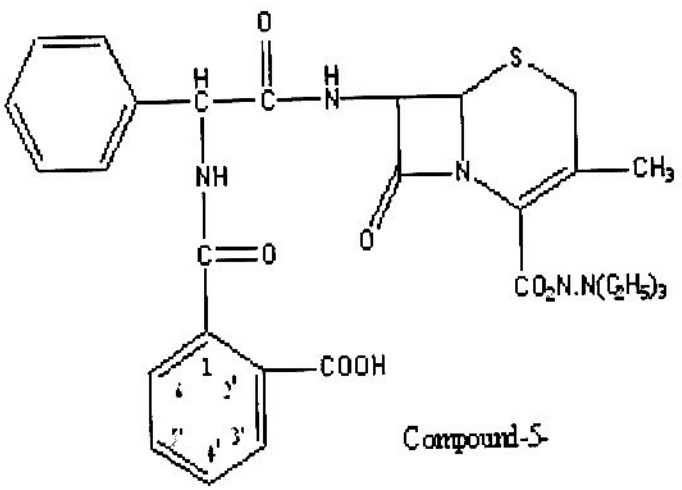
Scheme (2) The Structural Formula of Compounds



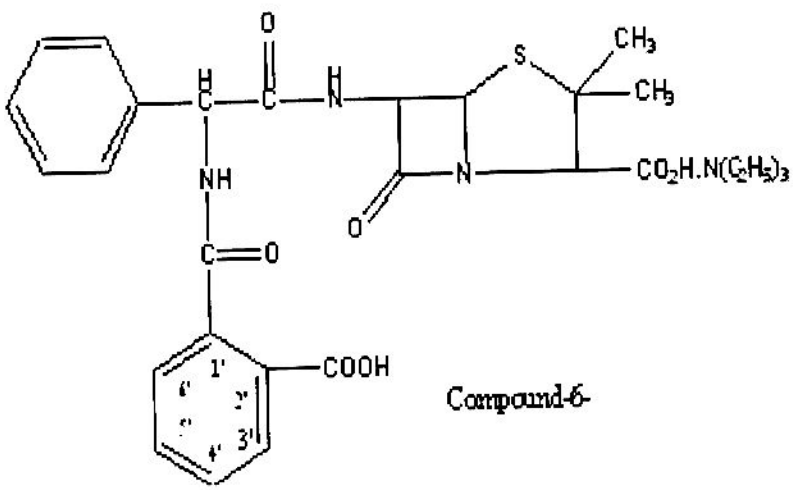
Compound-3-



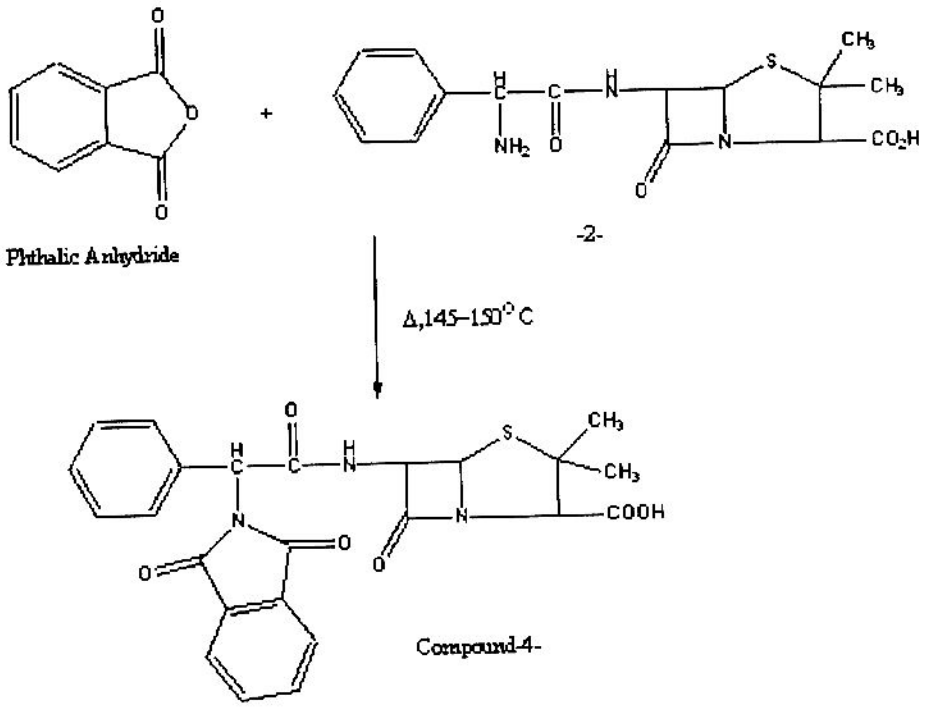
Compound-4-



Compound-5-



Compound-6-



Scheme -3- The reaction of Phthalic Anhydride with Ampicillin (2), to Form Compound -4-

Scheme: (4) The Reaction of Phthalic Anhydride with Ampicillin (2) at 0-10 °C at form Compound -6-

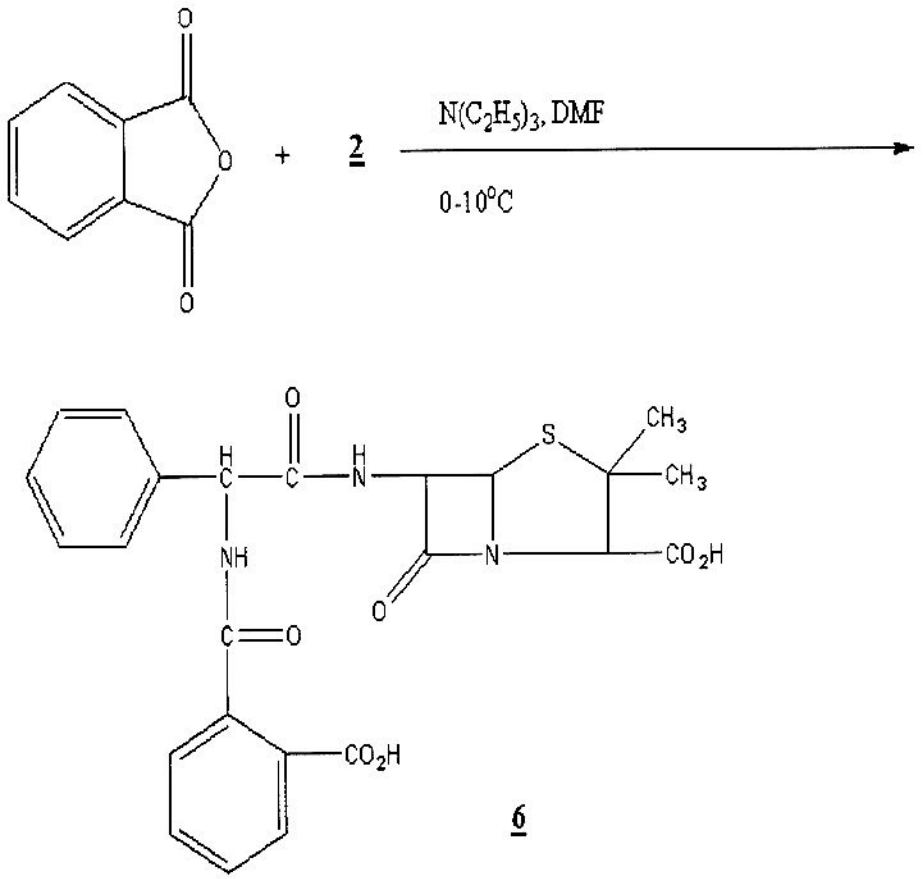


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Table: (1) The Physical data of Compounds (3-6)

Compound	Percent yield	Melting point		Rf value		
		Found	Reported	S1	S2	S3
3	62	150-152	-	0.71	0.75	0.25
4	65	161-163	-	0.70	0.73	0.16
5	30	140-143	-	0.80	0.78	0.32
6	35	170-172	-	0.75	0.65	0.25
Ampicillin	-	-	191-192	0.9	0.95	0.30
Cephalexin	-	-	185-187	0.85	0.9	0.31
Phthalic Anhydride	-	-	132-133	0.9	0.9	0.29

Solvent Systems

S1:Acetic acid : water :n-Butand (1:1:2)

S2:Acetone : Benzene : n- Butanol (1:3:1)

S3 : Chloroform : Ethanol (3:1)

Table: (2)C-H-N Analysis of Compounds (3-6)

No.	Chemical Formula	C-H-N					
		Found %			Calculated %		
		C	H	N	C	H	N
3	C24H19O6N3S	59.97	3.63	8.55	60.37	3.98	8.80
4	C24H21O6N3S	59.92	3.70	8.36	60.12	4.38	8.76
5	C30H36O7N4S	60.08	5.74	9.02	60.40	6.04	9.39
6	C30H38O7N4S	59.03	5.81	9.02	60.20	6.35	9.36

Table :(3) IR data of Compounds (3-6) Ampicillin and Cephalexin

Compound	C=O of β-lactam	C=O of carboxylic acid	C=O of amide	N_H bend of amide	N_H of amine
1	1770	1750	1650	1515	___
2	1780	1755	1645	1520	___
3	1775	1750	1650	1520	___
4	1770	1750	1650	1515	___
Ampicillin	1780	1750	1650	1520	1550
Cephalexin	1780	1755	1645	1520	1540

Table: (4) Antimicrobial activity of compounds (3-6)

(Zone of inhibition in m.m)

Compounds	Antimicrobial activity			
	Staph aureus	E-coil	Psed. araginosa	Condida
50 µg/ml				
3	16	15	_____	_____
4	11	12	_____	_____
5	17	14	_____	_____
6	13	11	_____	_____
Cephalixin	20	18	_____	_____
Ampicillin	15	14	_____	_____
100 µg/ml				
3	20	17	12	____
4	17	11	7	____
5	22	16	14	____
6	19	12	10	____
Cephalixin	25	20	_____	____
Ampicillin	21	14	_____	____

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تخليق مشتقات جديدة من البيتالاكتام ذي فعالية مضادة للميكروبات

مثنى ضاري سعود ، نضال فالح المجول ، عضيد خالد شراد *
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الخلاصة

تحضير مشتقات جديدة لمضادات حياتية من نوع بيتا لاكتام . لقد تمت الاستفادة من مجموعة الامين الاحادية الموجودة في هذه المركبات كموقع لتحضير المشتقات .

كان الهدف وراء ذلك تحضير مركبات جديدة ممكن ان تكون لها قابلية اكبر على مقاومة الانزيمات المحللة لهذا النوع من المركبات . ان هذه المركبات يمكن ان تكون مشاكلة للمركبات الاصلية ، كذلك فانها يمكن ان تكون مقدمات ادوية محتملة لتلك المركبات .