Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(23):51-56
(http://www.derpharmachemica.com/archive.html)

Synthesis, Characterization and Preliminary Antimicrobial Evaluation of New Derivatives of Ceftazidime

Shakir M Alwan*, Mohammed H Mohammed, Alaa A Alhassan

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq

ABSTRACT

Cephalosporins derivatives remain as one of the most versatile class of compounds against bacteria and therefore, are useful for further molecular exploration. The development of new derivatives of ceftazidime with improved activity against resistant microbes is of high potential. Chemical synthesis of four new derivatives of ceftazidime, in which amino acids was linked to ceftazidime by substitution of pyridine ring at C3 position gave the target derivatives A (1-4) as a first line. In addition, to the N-acylation of the free amine group of aminothiazole ring by acids chloride to obtain three target derivatives B (1-3) as a second line.

New ceftazidime derivatives were successfully prepared, characterized and identified using; melting point, TLC, spectral (FT-IR) and elemental microanalysis (CHNS). All target compounds A (1-4) and B (1-3) were evaluated for their antibacterial activity against certain bacteria such as; Escherichia coli and Pseudomonas aeruginosa, Streptococcus pneumoniae and Staphylococcus aurous. Generally, all seven new derivatives of ceftazidime showed significant antibacterial activity against the tested microorganisms, especially G (-) bacteria as compared to ceftazidime.

Keywords: Ceftazidime, Aminoacid, Acyl chloride, Antimicrobial activity, Pyridine

INTRODUCTION

Ceftazidime is a broad-spectrum third generation cephalosporin antibiotic that was exhibits activity against a wide range of Gram-negative and Gram-positive bacterial pathogens, including *Escherichia coli* and *Pseudomonas aeruginosa* [1].

It has oxime moiety and this more complex; containing two methyl groups and a carboxylic acid, this assemblage conveys even more pronounced β-lactamase stability, greater anti-pseudomonas activity and increase activity against gram-positive bacteria. The C-3 side chain replaced by a charged pyridinium moiety. The latter considerably enhances water solubility and highly activates the β-lactam bond toward cleavage [2].

The stability of a drug substance or drug product is described as its ability to remain within established pharmacopoeial specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods, under stated or reasonably expected conditions of storage and use [3]. β-lactams are known to be unstable in aqueous media (intrinsic fragility of the β-lactam ring) particular attention must be paid to this condition [4].

Like all cephalosporins, aqueous solutions of ceftazidime are however unstable and subject to hydrolytic degradation; indeed several studies have examined its stability under different conditions, its degradation kinetics influenced by various catalysts) buffers, carbohydrates, etc.) [5]. Some study found that ceftazidime in formulation of viscous eye drops degraded to form pyridine a potentially toxic substance [6].

The carboxyl group of β-lactam is of crucial importance for binding and later relative rearrangement of the antibiotic. The studies examined the molecular differentiation mechanisms between cephalosporins and penicillins in β-lactamases [7]. So that some cephalosporins have one carboxyl group such as cefadroxil, cephalexin and cephalothin but other contain two-carboxyl group as cefixime, cefotetan and ceftazidime.

The D-amino acids have increased in recent decades with the development of new analytical methods highlighting their presence in all kingdoms of life. Their involvement in physiological functions and the presence of metabolic routes for their synthesis and degradation.

Furthermore, D-amino acids are gaining considerable importance in the pharmaceutical industry [8].

Some cephalosporin compounds having amino acid derivatives. These facts prompt to investigate the introduction of a new series of cefaclor compounds containing modified acyl chains at the C-7 or amino acid amide residues at the C-4. The determination of the effect of these modifications on the antibacterial activity of cefaclor against gram-positive and gram-negative bacteria including some strains of *Pseudomonas aeruginosa* and proteus vulgaris, which are normally insensitive to some cephalosporin antibiotics [9].

Prodrugs of ceftizoxime containing an amino acid on the aminothiazole moiety synthesized and found with potential activity and improved physicochemical properties and oral absorption [10]. The present study designed to synthesis a number of new ceftazidime derivatives by two lines:

One is substitution reaction between ceftazidime and aminoacids when phenylalanine, proline, valine, and glutamic acid substitute with pyridine ring in ceftazidime molecule to yield four new compounds.

Other lines include N-acylation of 2-amino thiazole in ceftazidime by reaction of acetyl chloride, Chloroacetyl chloride, and benzoyl chloride with 2-amino thiazole of ceftazidime molecule to yield three new compounds.

MATERIAL AND METHODS

Melting point (uncorrected) were determined using electrical melting point apparatus, Electro-thermal 9300, USA. The infrared spectra were performed in KBr disc by FT-IR spectrophotometer/Shimadzu. Elemental micro-analysis (CHN) was performed by Vario micro, Germany. Checking the purity of the products as well as monitoring the progress of the reaction was achieved by thin layer chromatography using silica gel F_{254} aluminum sheets, Macherey-nagel, Germany.

CHEMICALS AND REAGENTS

L- Valine, Thomas-barker, India. L-glutamic acid, Riedel, Germany. L- Phenylalanine, Singopharm, China. L- Proline, Chemical point, Germany. Chloroacetyl chloride, Merk, Germany. Acetyl chloride, alfa-aesar Germany. Benzoyl chloride, BDH, England. Ceftazidime, L.D.P laboratory torlan, Spain.

Synthesis of series a compounds

A mixture of an accurately weighed amount of ceftazidime sodium (3.66 mmol, 2 g), aminoacid was listed in Table 1, and Triethylamine (7.23 mmol, 1 ml) was reflux for 5 in 40 ml dimethylformamide (DMF) at 60-70ºC. The mixture was left to stir overnight then filtered and the filtrate was evaporated. The resulted residue was washed with diluted HCl and the pH was adjusted to 4-5. The Precipitated compound was washed with D.W (3 × 50 ml), acetone and diethyl ether (1-4).

Table 1: List of the amino acids used

Amino acid	Amount	Product synthesized
L-Valine	7.3 mmol (0.857 g)	A1
L-Glutamic acid	7.3 mmol (1.07 g)	A2
L-Phenylalanine	7.3 mmol (1.2 g)	A3
L-Proline	7.3 mmol (0.85 g)	A4

The percent yield; physical appearance, melting point, and R_f- values of the synthesized compounds are listed in Table 2.

Synthesis compounds series B

A mixture of an accurately weighed amount of ceftazidime sodium (3.66 mmol, 2 g), and TEA (7.23 mmol, 1 ml) in D.W 20 ml of was cold to 0-5ºC. Then acyl chloride as listed in Table 3 was added dropwise to the above mixture with continues stirring. The mixture was left to stir overnight and the precipitated compound was washed with D.W (3 × 50 ml), acetone 50 ml and diethyl ether 25 ml. The percent yield; physical appearance, melting point, and R_f- values of the synthesized compounds are listed in Table 2 [1-3].

Antimicrobial evaluation

Antimicrobial activity evaluation done at laboratory the department of biology, college of science, University of Mustansiriyah [11,12].

The newly synthesized derivatives were tested for their antimicrobial activity by well diffusion method against the following

Table 2: The physical appearance, yield percent, uncorrected melting point, and R_F value of the synthesized compounds (A1-B3)

Compound	Chemical Formula	Physical Appearance	% yield	Melting Point ºC	R_f
A1	$C_{22}H_{28}N_6O_9S_2$ 2HCl	Pale yellow Powder	57	240 Decom.	0.35 (A)
A2	$C_{22}H_{26}N_6O_{11}S_2$ 2HCl	Deep brown powder	62	230 Decom.	0.42 (A)
A3	$C_{26}H_{27}N_6O_9S_2$ 2HCl	Pale brown powder	45	192 Decom.	0.24 (A)
A4	$C_{22}H_{25}N_6O_9S_2$ 2HCl	Yellow powder	50	245 Decom.	0.35 (A)
B1	$C_{24}H_{23}ClN_6O_8S_2$.HCl	Orange powder	60	180 Decom.	0.37 (B)
B2	$C_{24}H_{24}N_6O_8S_2$ .HCl	Pale yellow powder	58	173 Decom.	0.31 (B)
B3	$C_{29}H_{26}N_6O_8S_2$ .HCl	Brown to Yellow powder	43	168 Decom.	0.33 (B)
Ceftazidime Sodium	$C_{22}H_{22}N_6O_7S_2Na$	white	–	200-210 Decom.	0.52 (B)

Table 3: list of acyl choride used

Acyl chloride	Amount used	Product synthesized
Chloroacetyl chloride	14.64 mmol (1.16 ml)	B1
Acetyl chloride	14.64 mmol (1 ml)	B2
Benzoyl chloride	14.64 mmol (1.7 ml)	B3

microorganisms and at two concentrations (0.1 mg/ml), (0.05 mg/ml):

1-Gram-negative bacteria: *Escherichia coli* and *Pseudomnas aeruginosa*;

2-Gram-postive bacteria: *Staphylococcus aureus* and *Streptococcus pneumoniae*;

Type of agar used: Muller Hinton agar;

Time of incubation: 24 h at 37ºC.

Well-diffusion method

Ceftazidime used as reference for testing the anti-bacterial activity [13]. The synthesized compounds and reference dissolved in diluted DMF (1:3) two concentrations were used 0.1 mg/ml and 0.05 mg /ml respectively. for screening in vitro antibacterial activity against the selected gram positive and gram negative and screening antifungal activity against the selected fungus (candida albicans).

After we prepared stock solution and made several dilutions to obtained 0.1 mg/ml and 0.05 mg/ml then application of the samples and reference to plate seeded with 0.1 ml of 10^8 CFU/ml of bacteria and with 10^6 CFU/ml of fungal. Triplicates of each concentration for each microorganism species were prepared. The inoculated plates incubated at 37ºC for 24 hr.

The diameter of the inhibition zones for each of the newly synthesized and reference compounds against each of the tested microorganism are listed in Table 4.

Table 4: Antimicrobial evaluation of the synthesized compounds

Compounds	*Staphylococcus aureus*		*Staphylococcus pneumoniae*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Canadians albicans*	
Conc. DMF	**50 µg**	**100 µg**	**50 µg**	**100 µg**	**50 µg**	**100 µg**	**50 µg**	**100 µg**	**50 µg**	**100 µg**
Ceftazidime	36	37	22	27	34	25	34	33	35	34
A1	25	16	17	14	24	17	22	26	20	18
A2	14	15	10	17	15	16	12	15	12	19
A3	22	27	12	19	20	23	18	25	17	25
A4	14	27	9	21	17	29	13	19	10	26
B1	23	25	18	19	20	18	21	24	20	20
B2	25	30	20	22	26	20	24	27	22	30
B3	17	23	14	17	20	18	19	21	17	25

RESULT AND DISCUSSION

The designated compounds were synthesized according to schemes (1 and 2). A two series of compounds were synthesized, A (1-4) in which the pyridinium group of ceftazidime was replaced by different amino acids and series B (1-3) in which the amino-group of the 2-aminothiazole was acylated with different acyl moiety. The physical properties of the synthesized compounds are shown in Table 2. The structures of synthesized compounds were characterized by elemental microanalysis and infrared spectroscopy.

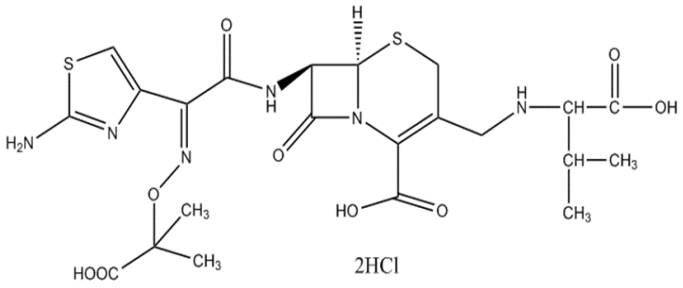
The using of two solvent systems A and B to differentiate between reactants and products and to follow progress of reactions.

A=Methanol: Acetone: Water (4:0.5:0.5);

B=Methanol: water (4:1);

As illustrated on Table 2.

Compound A1 2-aminothiazol-4-yl-2 (2-carboxypropan-2-yl) oxyimino acetamido-3- (1-carboxy-2-methylpropyl) amino methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid dihydrochloride.

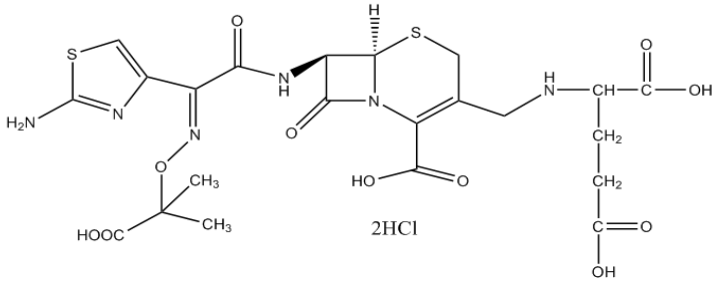


The physical parameters and percent yield in Table 2 and The IR characteristic bands 3408-3313(N-H symmetric and asymmetric stretching vibration of primary amines), 1757)C=O stretching vibration of β-lactam ring) 1710)C=O stretching vibration of –COOH groups) 1624) C=O stretching vibration of amide group) and CHN analysis.

Calculated; C: 40.19, H: 4.60, N: 12.78, S: 9.75;

Found C: 40.86, H: 5.13, N: 13.95, S: 9.472;

Compound **A2** 2-aminothiazol-4-yl-2-(2-carboxypropan-2-yl) oxyimino acetamido)-2-carboxy-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-3-yl) methyl)-L-glutamic acid dihydrochloride.

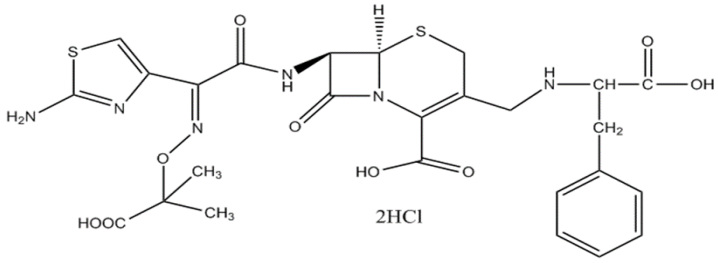


The physical parameters and percent yield in Table 2 and The IR characteristic bands 3298-3200 (N-H symmetric and asymmetric stretching vibration of primary amines), 1764)C=O stretching vibration of β-lactam ring) 1712)C=O stretching vibration of –COOH groups) 1651) C=O stretching vibration of amide group). The CHNS analysis:

Calculated: C: 38.43, H: 4.11, N: 12.22, S: 9.33;

Found C: 39.33, H: 4.262, N: 13.08, S: 10.31;

Compound A3 2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yl) oxyimino) acetamido)-3-(1-carboxy-2 phenylethyl) amino methyl) -8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid dihydrochloride.

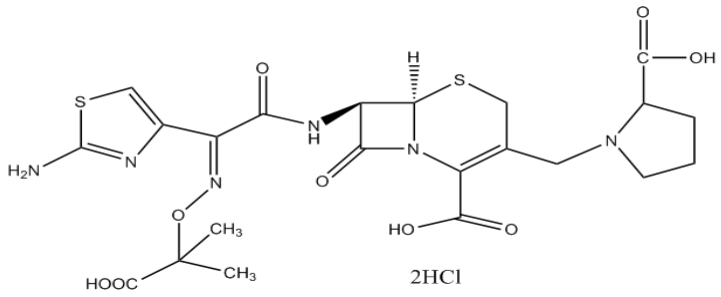


The physical parameters and percent yield in Table 2 and The IR characteristic bands 3388-3263(N-H symmetric and asymmetric stretching vibration of primary amines), 1757)C=O stretching vibration of β-lactam ring) 1708)C=O stretching vibration of –COOH groups) 1620) C=O stretching vibration of amide group). The CHNS analysis:

Calculated C: 44.26, H: 4.29, N: 11.91, S: 9.09;

Found C: 45.37, H: 3.33, N: 11.91, S: 7.939;

Compound A4 2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yl) oxyimino-acetamido-3-((2-carboxypyrrolidin-1-yl)methyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid dihydrochloride.

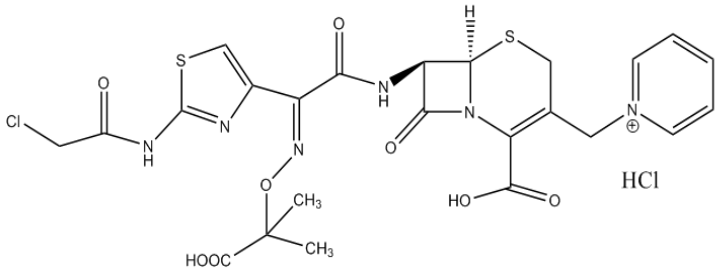


The physical parameters and percent yield in Table 2 and The IR characteristic bands 3396-3309 (N-H symmetric and asymmetric stretching vibration of primary amines), 1757) C=O stretching vibration of β-lactam ring) 1708)C=O stretching vibration of –COOH groups) 1620) C=O stretching vibration of amide group). The CHNS analysis:

Calculated C: 40.31, H: 4.31, N: 12.82, S: 9.78;

Found C: 41.85, H: 5.073, N: 13.48, S: 9.87;

Compound B1 2-carboxy 2-(2-carboxypropan-2-yl)oxyimino-2-(2-(2-chloroacetamido)thiazol-4-yl)acetamido-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridin-1-ium hydrochloride.

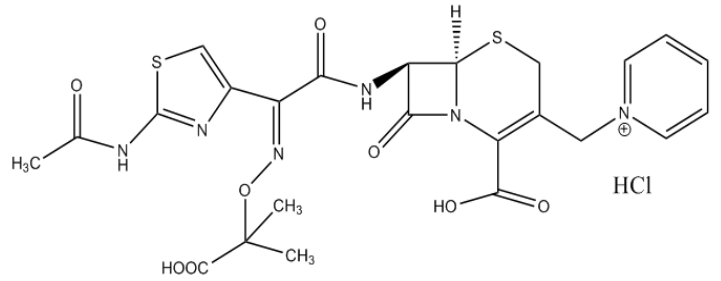


The physical parameters and percent yield in Table 2 and The IR characteristic bands 3282 (N-H stretching vibration of secondary amides), 1776 (C=O stretching vibration of β-lactam ring) 1708)C=O stretching vibration of –COOH groups) 1658) C=O stretching vibration of amide group). The CHNS analysis:

Calculated: C: 43.64, H: 3.82, N: 12.72, S: 9.71;

Found C: 42.57, H: 4.73, N: 13.5, S: 10.244;

Compound B2 2-(2-acetamidothiazol-4-yl)-2-(((2-carboxypropan-2-yl)oxy)imino)acetamido)-2-carboxy-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-3-yl) methyl) pyridin-1-ium hydrochloride.

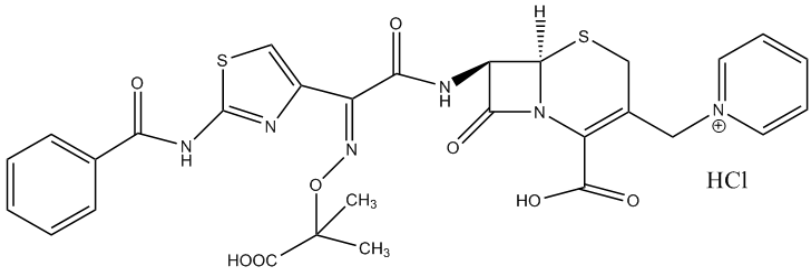


The physical parameters and percent yield in Table 2 and The IR characteristic bands 3277 (N-H stretching vibration of secondary amides), 1778)C=O stretching vibration of β-lactam ring) 1712)C=O stretching vibration of –COOH groups) 1631) C=O stretching vibration of amide group). The CHNS analysis

Calculated C: 46.04, H: 4.19, N: 13.42, S: 10.24

Found C: 45.13, H: 4.95, N: 13.86, S: 10.318

Compound B3 2-(2-benzamidothiazol-4-yl)-2-(((2-carboxypropan-2-yl)oxy)imino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-en-3-yl)methyl)pyridin-1-ium hydrochloride.



The physical parameters and percent yield in Table 2 and The IR characteristic bands 3307 (N-H stretching vibration of secondary amides), 1766)C=O stretching vibration of β-lactam ring) 1710)C=O stretching vibration of –COOH groups) 1658) C=O stretching vibration of amide group). The CHNS analysis:

Calculated C: 50.62, H: 4.10, N: 12.21, S: 9.32;

Found C: 49.64, H: 4.37, N: 11.89, S: 10.56.

Preliminary antimicrobial evaluation

The synthesized compounds were subjected to antimicrobial evaluation by well-diffusion method. The inhibition zone (mm) was measured in comparison with ceftazidime.

CONCLUSION

A series of new derivatives of ceftazidime have been synthesized successfully in acceptable yields and screened for their antimicrobial activity against bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus pneumoniae*) and fungal strain (*Canadians albicans*)

It concluded that the new derivatives of ceftazidime linked with certain aminoacids were found to possess moderate antimicrobial activities. Furthermore, the compounds B2 and A3 have significant activity against selected gram +ve, gram –ve and fungi.

REFERENCE

[1] Bian, Lei, N. Bushby, *Journal Labelled Compounds Radiopharmaceuticals.*, **2015**, 58(7), 313-316.

[2] Foye, *Foye's Principles of Medicinal Chemistry*., 6th edition, Edited by T.L. Lemke, D.A. Williams, L.W. Wilkins, **2008**, 1028-1130

[3] I. Ivanovic, L. Zivanovic, M. Zecevic, *J. Chromatogr.,* **2006**, 1119, 1209-1215.

[4] Servais, Hélène, M. Paul, Tulkens, *Antimicrobial Agents Chemotherapy.*, **2001**, 45(9), 2643-2647.

[5] A. Farina, R. Porra, V. Cotichini, A. Doldo, *J. Pharmaceut. Biomed. Anal.,* **1999**, 20(3), 521-530.

[6] Stendal, L. Tove, W.E.N.D.Y. Klem, H.H. Tønnesen, I. Kjønniksen, *Am. J. Health. Syst. Pharm*.**, 1998,** 55, 683-685.

[7] F. Ferrer, Cristina, J. Frau, J. Donoso, F. Muñoz, *Proteins: Structure, Function, Bioinformatics.*, **2003**, 51(3), 442-452.

[8] M. Rodríguez, Sergio, A.I. Martínez-Gómez, F. Rodríguez-Vico, J.M. Clemente-Jiménez, L. Heras-Vázquez, F. Javier, *Chem. Biodiver.,* **2010**, 7(6), 1531-1548.

[9] H.M. Hassan, S.A. Shedid, M.F. Badie, R.M. Eisawy, *J. Am. Sci.,* **2011**, 7(1), 215-221.

[10] M. Nobuhiro, T. Kodama, A. Sakai, T. Suzuki, T. Sugihara, S. Yamaguchi, T. Nishijima, *Int. J. Antimicrob. Agents.,* **2001**, 18(5), 451-461.

[11] H. Mohamed, A.H. Bayoumi, K.M. El-Gamal, A.S. Mayhoub, H.S. Abulkhair, *Beni-Suef. Univ. J. Basic. Applied. Sci.,* **2015**, 4(4), 338-345.

[12] S. Parmar, A. Patel, M. Mistry, M. Champaneriya, *RJPBCS*., **2012**, 3(3), 996-1003.

[13] Balouiri, Mounyr, M. Sadiki, S.K. Ibnsouda, *J. Pharmaceut. Anal.*, **2016,** 6(2), 71-77.