Synthesis of new derivatives of Ceftazidime as possible Prodrugs

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Abstract

Five new ceftazidime derivatives were designed and synthesized in an attempt to improve the acid stability and may increase the spectrum of ceftazidime. The synthesized compounds included; Schiff base of ceftazidime (compound 1), ceftazidime lysine amide Schiff base (compound 2), ceftazidime lysine amide (compound 3), ceftazidime-di-lysine amide Schiff base (compound 4) and ceftazidime-di-lysine amide (compound 5). New ceftazidime derivatives were successfully prepared characterized and identified using spectral and elemental microanalysis (CHNS) analyses and the results comply with the calculated measurements.

Compounds 1 and 2 were subjected to a stability study in phosphate buffer (0.2M, pH 7.4) and in KCl/HCl buffer (0.2M, pH 1.2) at different time intervals (0 – 240 min) incubated at 37 °C. This revealed that both compounds in phosphate buffer (0.2M, pH 7.4) are significantly stable with $t_{1/2}$ of 18hrs and 24hrs respectively. However, compounds 1 and 2 are less stable in KCl/HCl buffer (0.2M, pH 1.2) with $t_{1/2}$ of 3.48hrs and 3.13hrs respectively.

The antibacterial evaluation of the new ceftazidime derivatives showed various degrees of antibacterial activities when compared with ceftazidime. The chemical modifications of ceftazidime showed slight effect on activities and most of compounds retained the antibacterial activities. Compounds 2 and 4 afforded comparable antibacterial action. However, compounds 3 and 5 were equipotent with ceftazidime with respect to E.coli and Staph. aureose. Compound 4 has better activity than ceftazidime with respect to Pseudomonas aeruginosa. Schiff's base derivative of lysine (2, 6-bis-(benzylideneamino) hexanoic acid) gave a reasonable antibacterial action towards Escherichia coli and Streptococcus Spp; as compared with lysine which has no antibacterial activity.

Key words: Ceftazidime, Schiff bases, Lysine.

تخليق ودراسة الفعالية البكتيرية لمشتقات جديدة لعقار السفتازديم

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الخلاصة

تم تصميم وتحضير خمسة مشتقات جديدة لعقار السفتازيديم تحوي على قواعد شف وقواعد شف للاسين وثنائي اللايسين في محاولة لزيادة الفعالية البايولوجية وتحضير مشتقات تعطى عن طريق الفم. تضمنت المركبات المحضرة قواعد شف للعقار السفتازيديم(مركب١) وسفتازيديم لايسين امايد قاعدة شف (مركب ٢) وسفتازيديم لايسين امايد (مركب ٣) وسفتازيديم-ثنائي الايسين-قواعد شف (مركب٤) وسفتازيديم –ثنائي اللايسين (مركب ٥). حضرت المشتقات الجديدة بواسطة تفاعل مجموعة الامين التابعة لمجموعة الامينوثيازول والعقار المذكور مع البنزلدهايد لتكوين قواعد شف (مركب ١) .تضمن البحث كذلك تفاعل اللايسين مجموعتي الامين الاحادي مع البنزلدهايد لتكوين قواعد شف للايسين والذي تم ربطه بمجموعة الامين التابعة للأمينوثيازول بواسطة آصرة أمايد لتكوين قواعد شف للايسين المرتبطة بالسفتازديم (مركب ٢). كما تم تحضير سفتازديم المرتبط باللايسين (مركب ٣) بواسطة تحلل مجموعة قواعد شف بواسطة حامض الهيدروكلوريك. كما تم تحضير سفتازديم مرتبط بثنائي اللايسين الحاوي على قواعد الشف بواسطة اصرة الامايد لتكوين (مركب ٤). وبطريقة مشابهة اجريت عملية تحلل لمجموعة قواعد شف لمركب ٤ بواسطة حامض الهيدروكلوريك لتكوين (مركب ٥) .

تم تحضير المركبات بشكل ناجح وتم تشخيص التركيبه الكيمياوية للمركبات الجديدة بواسطة الطرق الطيفية وتحليل العناصر ودرجة الانصهار والتحليل بواسطة كروماتوكرافيا الرقائق وجاءت النتائج مطابقة للتركيبة المقترحة. المركبين ١ و٢ في محاليل البفر الفوسفات والبوتاسيم اظهروا درجة امتصاص في ٢٩٩ و٢٧٧ ن.م. بالتتابع والتي تختلف عن درجة امتصاص السفتازيديم(٢٥٤ ن.م.), درجة ثبات المركبان (١ و ٢) في محاليل بفر فوسفات الصوديوم (0.27 M, pH 7.4) الدارئة وكذلك محلول كلوريد البوتاسيوم وحامض الهيدروكلوريك (0.2M pH 7.4). اظهرت الدراسة درجة ثباتية واضحة ومعقولة بالنسبة للمركبين اعلاه في محلول الفوسفات وكانت درجة ثباتية المركبين اقل بالنسبة للمحلول الاخرلفترات زمنية (٠-٢٤٠دقيقة) ودرجة حرارة ٣٧ درجة مؤية.كما تم تقييم الفعالية المضادة للبكتريا مع انواع مختلفة من البكتريا كما موضح في الجداول وكانت النتائج مناسبة، حيث اظهرت معظم المركبات فعالية مقبولة وجيدة مقارنة مع عقار السيفتازيدم.

كانت نتائج تقييم الفعالية للمكربين (٢ و ٤) متقاربة مع عقار السفتازيديم ,اما نتائج تقييم فعالية المركبين (٣ و ٥) فكانت متقاربة مع السفتازديم فيما يخص بكتريا Escherichia coli وبكتيريا auroseStaphylococcus.. (مركب٤) لديه فعالية مثلى ضد بكتيريا Pseudomonasaeruginosa. اما(المركب٢) الذي يمثل قواعد شف للايسين وكانت نتائجه مقبولة وجيدة ضد بكتريا Escherichia coli و Streptococcus pyogene وهذا افضل بكثير من اللايسين لوحده الذي ليس له فعاليه ضد هذه الانواع من البكتريا.

الكلمات المفتاحية: السفتازيديم ، قواعد شف ، اللايسين .

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Introduction

Chemical modifications of the C-3 carboxyl group and the C-7 amino group in the cephem nucleus produce various semi-synthetic cephalosporins that have different antibacterial spectra, β-lactamase sensitivity/resistance and pharmacokinetic properties [(1)]. Most of the known cephalosporins are prepared for parental administration, and still there is a great need for parental cephalosporin that has β-lactamase-resistant and anti-pseudomonal activity [(2)]. Oral activity conferred by the phenylglycyl substituent is attributed to increased acid stability of the β- lactam ring, resulting from the presence of a protonated amino group on the C-7 acylamino portion of the molecules. Carrier mediated transport of the dipeptide-like and zwitterion cephalosporin are important factors in achieving oral absorption [(3)]. A series of monofunctional and bifunctionalprodrugs of Cefizoxime was synthesized and shown to have improved physicochemical properties and oral absorption [(4)]. Ceftazidime is a semi-synthetic, β-lactam antibiotic containing a pyridinium ring and used for parenteral administration. Ceftazidime is stable to most β-lactamases produced by G (+) and G (-) organisms, and consequently is active against many ampicillin and cephalothin resistant strains, except methicillin resistant strains [(5)].

Schiff bases have been shown to exhibit a wide range of biological activities including antimicrobial [6,7], anti-inflammatory [(8,9)], analgesic [(9)], anti-tubercular[(10-12)], antimycobecterial[(13,14)], antioxidant [(15)], antiviral [(16)] and enzyme inhibitors [(17)].

Lysine based prodrugs are widely used for different purposes, such as targeting certain drugs to a specific site and improve pharmacokinetic properties. L-lysine linked with amphetamine was found useful for treatment of Attention Deficit Hyperactivity Disorder [(18)]. L-lysine was linked with aminoflavon through an amide bond in order to improve pharmacokinetic properties affording marked antitumor effect [(19)]. A new series of 8-aminoquinolons derivatives prepared by conjugation with amino acids, dipeptides and pseudo-dipeptide have been synthesized and showed promising cytotoxic, anti-leshmenial and broad spectrum of antifungal activities [(20)]. L-lysine linked to Cefizoxime resulted in significant improvement in oral absorption [(4)]. A prodrug of cephalosporin (RWJ-333441) was prepared by linking lysine, through an amide bond, resulted in an improvement in aqueous solubility that is useful for parenteral administration [(21)]. Di/tripeptides and peptide-like drugs are absorbed across the brush-border membrane (BBM) by H^+ - coupled peptide transporter (PEPT1), intracellular peptides and peptide-like drugs exit from cells across the basolateral membrane (BLM) by an unidentified peptide transporter [(22).]

In an attempt to produce new derivatives of ceftazidime that may have broader spectrum of activities, acid stable and could be used orally, a new series was designed and synthesized, as new Schiff bases of ceftazidime and ceftazidime linked L-lysine Schiff bases and ceftazidime-linked di-lysine Schiff bases. Ceftazidime is also designed to be linked with lysine and di-lysine through amide bonds and these derivatives may target the amino acid and dipeptide transport systems and may provide an approach of producing an oral ceftazidime preparation.

Experimental

General

Melting points were determined (uncorrected) by using electrical melting point apparatus, Electro-thermal 9300, USA. Recording of the infrared spectra were performed in KBr disk using FT- IR spectrophotometer/Shimadzu. Elemental micro-analyses were performed by Euro-vector EA 3000A. UV Spectra were recorded by UV Spectrophotometer type AnalytikjenaSpecord 40/ Germany. The ascending TLC was run on silica gel F-254 (type 60) pre-coated aluminum sheets, for checking the purity of the products as well as monitoring the progress of the reaction.

Chemical synthesis

Five derivatives of ceftazidime (compounds 1-5) were designed and synthesized as Schiff bases of ceftazidime, Schiff bases of L-lysine linked to ceftazidime, L-lysine and di-lysine linked to ceftazidime that may improve pharmacokinetic properties and few could be applied for oral administration of ceftazidime and may broaden the antibacterial spectrum. Benzaldehyde was used to prepare the Schiff bases.

Synthesis of a new Schiff base of ceftazidime sodium (compound 1)

Disodium mono(7-((Z)-2-(2-((E)-benzylideneamino)thiazol – 4 – yl) – 2-(2-carboxylato propan – 2 – yl – oxyimino) -8-oxo-3-(pyridinium – yl – methyl) – 5-thia-1-azabicyclo(4.2.0) oct –ene – carboxylate).bicarbonate.

This derivative was prepared by reaction of ceftazidime sodium and benzaldehyde in methanol at 60 °C [(23)].

Benzaldehyde (1.56 mmol, 0.18g) in methanol (10 mL) was added to ceftazidime (1.56 mmol, 1g) dissolved in methanol (100 mL) and to the above solution TEA (1.56nmol, 0.48mL) was added. The mixture was refluxed for 6 hrs and was then acidified with (0.1N) HCl and filtered to remove the unreacted ceftazidime. The precipitate was washed with (2x30 mL) of distilled water and was dissolved in a solution of sodium bicarbonate solution (5%, 20mL) and dry acetone (60mL) was added and the solution was placed in a refrigerator to precipitate the product as yellowish-brown sodium salt of the Schiff base of ceftazidime. The physical appearance, percent yield and Rf values are listed on table 1. The IR characteristic bands (ν, cm^{-1}) are recorded as; 3191 (N-H stretching of secondary amid), 1745 (carbonyl of β-lactam), 1647 (carbonyl stretching of carboxylate) and 1585 (C=N stretching). Elemental microanalysis of compound 1 was listed on Table 2.

Synthesis of Schiff base of lysine, 2, 6-bis (benzylidene amino) hexanoic acid (2a)

The two aliphatic amino groups of lysine were reacted with benzaldehyde to form Schiff' bases [24]. Schiff' bases were collected as solids and characterized by chemical and spectral analysis. Lysine mono hydrochloride (26.3 mmol, 4.8g) was dissolved in distilled water (35ml) and NaOH (26.3 mmol, 1g) was added and the mixture was cooled in an ice bath. Benzaldehyde (52.6 mmol, 5.6 g) was added drop wise and the mixture was stirred for 2 hrs at room temperature. The obtained solid was excessively washed with distilled water and was recrystallized from methanol: ether (1:4) to afford a white needle-like crystals of the lysine Schiff' bases (2a). The physical appearance, percent yield and Rf values are listed on table (1). The IR characteristic bands (ν, cm^{-1}); 3251 (O-H stretching of carboxylic acid), 3082, 3028 (C-H aromatic stretching), 1703 (carbonyl group of carboxylic acid) and 1649 (C=N stretching).

Synthesis of new Schiff bases of ceftazidime- lysineamide disodium (compound 2)

Disodium mono (7-((Z)-2-(2-(2,6-bis((E)-benzylidene amino) hexaneamido)thiazol-4-yl)-2-(2-carboxylato propan-2-yl-oximino)acetamido)-8-oxo-3-(pyridinum-1-ylmethyl) -5-thia-1-azabicyclo(4.2.0)oct-2-ene carboxylate) bicarbonate.

The synthesis of compound (2) was achieved by reaction of compound 2a (lysine Schiff base) and ceftazidime by the mixed anhydride method [25], and as stated below.

Schiff base of Lysine 2a (3.41mmol, 1.098gm) was suspended in (75 ml) of a mixture of dry acetone and DMF (1:2). To the above suspension, TEA (3.41mmol, 0.48mL) was added and the mixture was placed in an ice bath at (-5 to -10C°). A solution of ethylchloroformate, ECF (3.41mmol 0.33ml) was added drop wise over a period of 10 min with continuous stirring, which was continued for further 30 min at room temerature. Ceftazidime (3.41mmol, 2.171gm) was dissolved in distilled water (20ml) containing TEA (3.41mmol, 048mL) and the solution was cooled to (0°C) and was added at once to the above mixture containing ECF with vigorous stirring for 4 hrs. The solvent was then evaporated and the resultant precipitate was washed with (0.1N) HCl. The suspension was filtered and the precipitate was collected and washed with ethanol (99.5%) to afford the hydrochloride salt. This was re-dissolved in a solution of sodium bicarbonate (25mL, 5%) and the pH of the solution was adjusted to 8. A cold dry acetone (60 mL) was added and the solution was placed in a refrigerator to precipitate compound 2 as sodium salt. The physical appearance, percent yield and Rf values are listed on table (1). The IR characteristic bands (ν, cm^{-1}); 3292 (N-H amide stretching), 1750 (carbonyl group of β-lactam), 1660 (carbonyl group of carboxylate), 1620 (C=N stretching of imine) and 1573 (stretching of carbonyl of carboxylate anion). Elemental microanalysis of compound 1 is listed on Table 2.

Synthesis of ceftazidime-lysineamide disodium (compound 3)

Disodium mono (7-((Z)-2-(2-carboxylatopropan-2-yloxyimino) 2(2)-2,6-diaminohexane amido) thiazol-4-yl) acetamido)-8-oxo-3-(pyridinuim-1-ylmethyl)-5-thia-1-aza bicyclo (4,2,0)oct-2-ene-2-carboxylate)bicarbonate.

This compound was obtained by cleavage of the Schiff' bases of compound 2 by reaction with hydrochloric acid solution at pH 2 incubated at 5-10˚C [26], as shown in scheme (2). Compound 2 was dissolved in distilled water (20mL), placed in an ice bath at (10°C) and a cold solution of HCl (1N, 10mL) was added drop wise to adjust the pH to 2 and the mixture was stirred for 4hrs. Cold acetone (40 mL) was added to precipitate compound 3 and the precipitate was collected and washed with ethanol to afford a fine white powder of the hydrochloride salt. The IR characteristic bands (ν, cm^{-1}); 3325, 3273 (N-H stretching

of primary aliphatic amine), 3197 N-H (carbonyl group of β-lactam), 1665 (carbonyl group of carboxylate anion) and 1614 (N=C stretching of imine). Elemental microanalysis of compound 3 is listed on Table 2.

Synthesis of ceftazidime-di-lysineamideSchiff bases di sodium (compound 4)

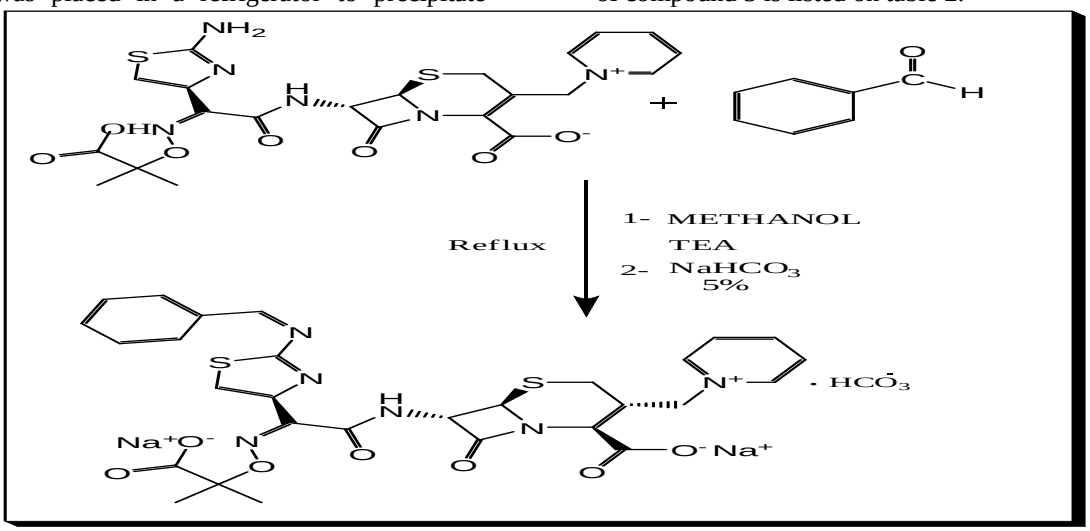
Disodium mono (7-((Z)-2-((2-(2-((E)-benzylideneamino)-(hexanamido) hexanamido) thiazol-4-yl)-2-(carboxylate propan-2-yl oxyimino) acetamido)-8-oxo-3-(pyridinum-1-ylmethyl)-5-azabicyclo (4,2,0)oct-2-ene-2-carboxylate) bicarbonate.

The synthesis of this compound was achieved by reaction of compound 2a with compound 3 using the following procedure, as shown in scheme (2).Schiff base of Lysine 2a (3.41mmol, 1.098gm) was suspended in a mixture (75 ml) of dry acetone and DMF (1:2), and to the above mixture TEA (3.41mmol, 0.48mL) was added and placed in an ice bath at (-5 to -10°C). A solution of ECF (3.41mmol 0.33ml) was added over a period of 10 min and the mixture was continuously stirred for further 30 min at room temperature. Compound 3 (1.705mmol, 1.226gm) was dissolved in distilled water (20ml) containing TEA (1.705mmol, 0.238mL) and was cooled to 0 °C and added at once to the solution of ECF-Schiff base of lysine and the mixture was vigorously stirred for 4 hrs. The solvent was evaporated and the resultant precipitate was suspended with diluted HCl and was filtered and the precipitate was collected as the hydrochloride salt, which was washed with ethanol. The precipitate was dissolved in a solution of sodium bicarbonate (5%, 15mL) and the pH of the solution was adjusted to 8. A cold dry acetone (60 mL) was added and the mixture was placed in a refrigerator to precipitate (stretching of secondary amide), 1758 compound 4, as sodium salt. The physical appearance, percent yield and Rf value are listed on table (1). The IR characteristic bands (ν, cm^{-1}); 3327 (N-H stretching vibration), 1758 (carbonyl group β-lactam), 1678 (carbonyl group stretching of carboxylate anion) and 1626 (C=N stretching of imine). Elemental microanalysis of compound 3 is listed on Table 2.

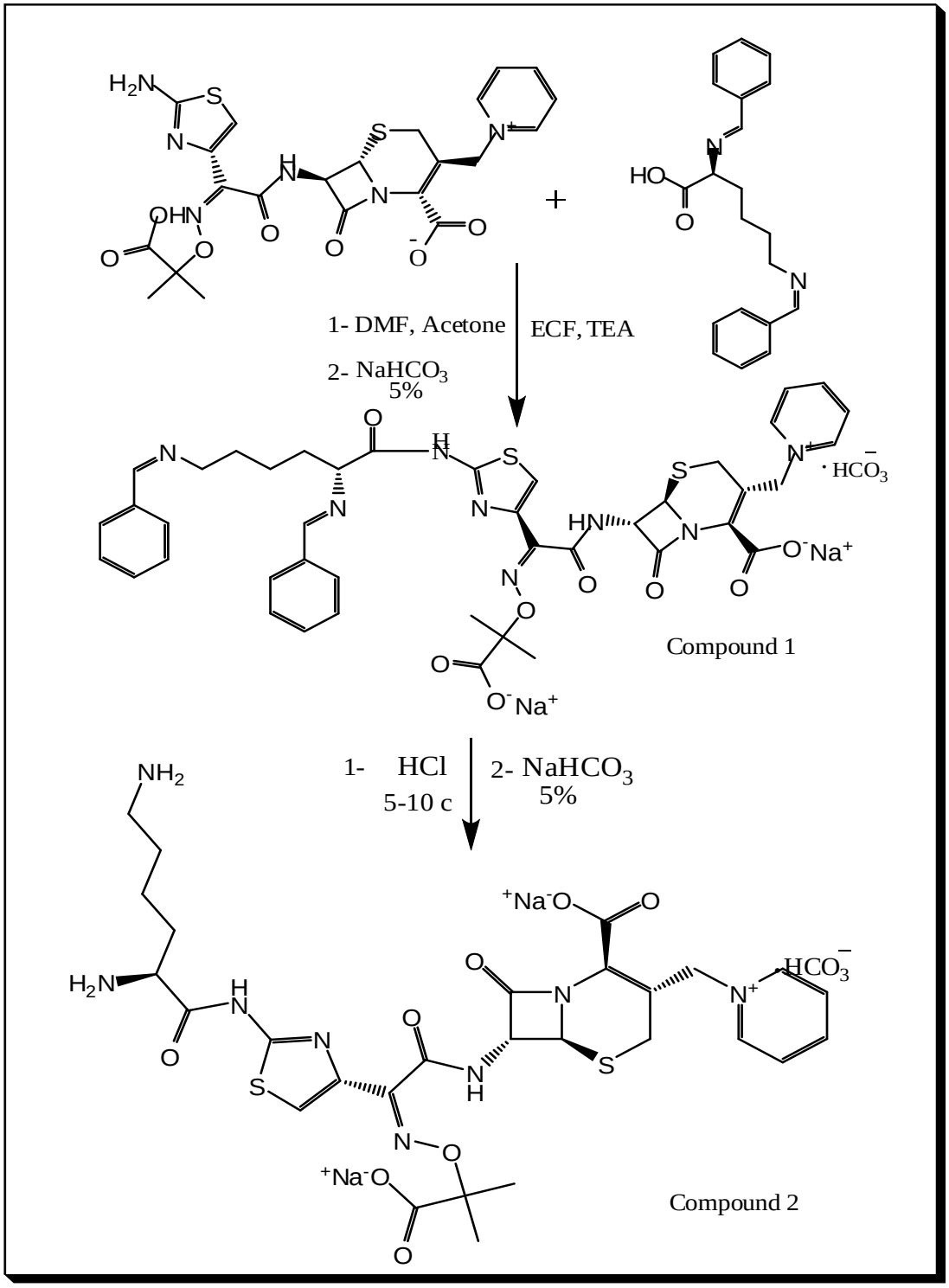
Synthesis of ceftazidime-di-lysineamide di sodium (compound 5)

Disodium mono(7-((Z)-2-(2-(R-2,6-bis(2,6-diaminohexanamido)-thiazol-4-yl)-2-(carboxylate-propan-2-yl-oxyimino)acetamido)-8-oxo-3-(pyridinium-1-yl-methyl)-5-thia-1-azabicyclo(4,2,0)oct-2-carboxylate) bicarbonate.

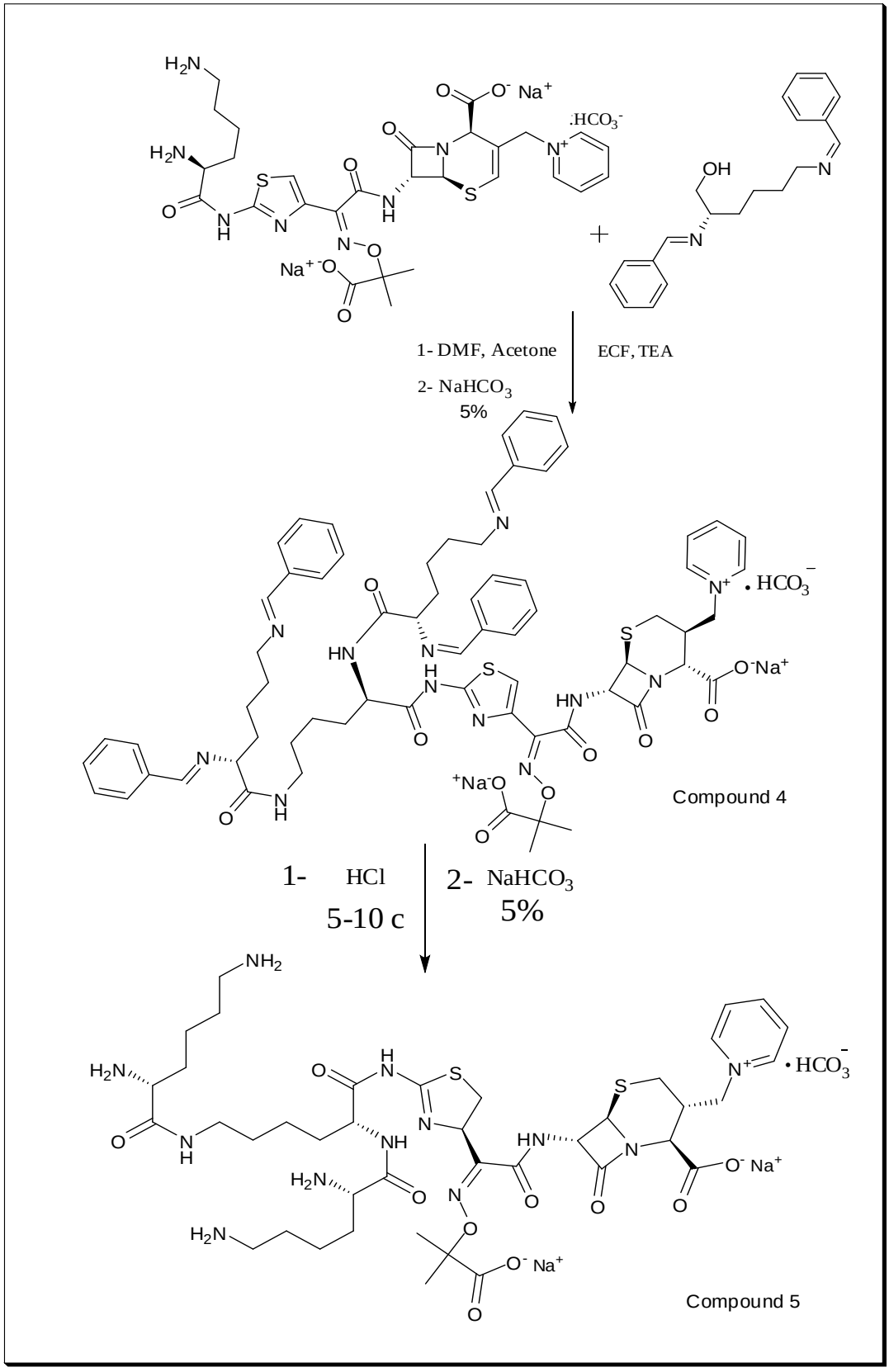
This derivative was prepared by reaction of compound 4 with hydrochloric acid solution at pH 2 and the mixture was incubated at 5-10 °C. Compound 4 (1.08 mmol, 1.5gm) was dissolved in distilled water (20mL) and placed in an ice bath at 5-10°C. A cold solution of hydrochloric acid (1N, 10mL) was added drop wise and the pH was adjusted to 2. The mixture was stirred for 4 hrs and was filtered and the product was separated as the dihydrochloride salt in the filtrate. Compound 5 as the dihydrochloride salt was neutralized by sodium bicarbonate (5%) and the pH of the solution was adjusted to 8. A cold dry acetone (60mL) was added to precipitate compound 5 as the sodium salt. The physical appearance, percent yield and Rf value are listed on table (1). The IR characteristic bands (ν, cm^{-1}); 3329, 3226 (N-H stretching of primary aliphatic amine), 3194 (N-H stretching of amide), 1759 (carbonyl group of β-lactam) and 1622 (C=N stretching of imine). Elemental microanalysis of compound 3 is listed on table 2.



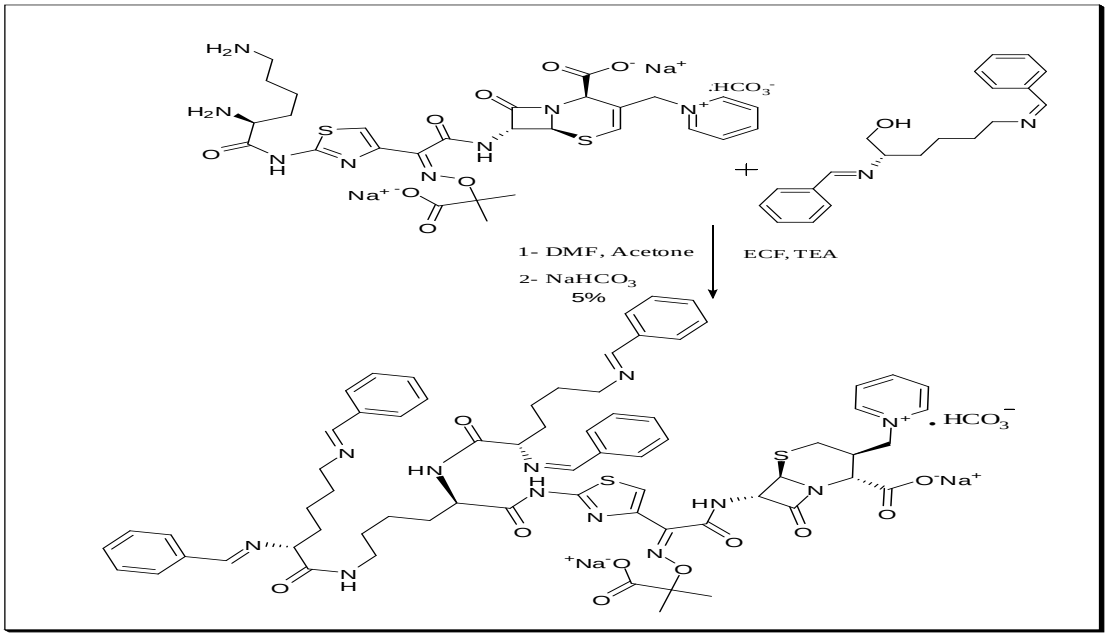
Scheme 1. Synthesis of Schiff base of ceftazidime sodium (compound 1).



Scheme 2. Synthesis of compounds 2 and 3.



Scheme 3. Synthesis of compounds 4 and 5.



Scheme 4. Synthesis of compound 4.

Results and Discussion

The IR characteristic bands shown in the spectra of compounds were used to identify and confirm their structures depending on the appearance and disappearance of certain chemical groups and consequently their bands [27, 28]. The IR spectra of the synthesized compounds showed characteristic bands of absorption, which were consistence with the proposed structures of the compounds. The IR spectrum of compound 1 (Schiff base of ceftazidime) showed characteristic bands and are previously listed. The disappearance of the amine band of the aminothiazole moiety in the IR spectra gives a good indication for the formation of the newly synthesized amide bond [29]. The newly synthesized compounds (1,2 and 4) contain an extra imine bond with regard to the already existing imines, as shown clearly in their chemical structures. However, the presence of such number of imines resulted in very close bands in the IR spectra of these compounds. The IR characteristic bands for compound 2a (Schiff base of lysine) showed imine group at 1649 cm^{-1}, which don't exist in lysine and at the same time, disappearance of the primary amine group in the region 3400-3300 cm^{-1} . Ceftazidime contain a primary aromatic amine of the aminothiazole moiety which appears at 3500-3400 cm^{-1} , while, compounds 3 and 5 contain primary aliphatic amine groups and their IR absorption bands appear at (3300-3200cm^{-1}). The IR spectra of (C=O) of carboxylic acid of ceftazidime, which appeared at 1705 cm^{-1} , while the asymmetric vibration of (COO-) of these compounds appeared at (1647-1678 cm^{-1}).

Elemental microanalyses were performed for the target compounds (1-5) to confirm their basic chemical formulas. The results were presented on table (2). The percent deviations of the observed to the calculated values were found to be acceptable and within the range of 0.4%.

Stability of compounds 1 and 2 in aqueous buffer solutions

UV spectra of the aqueous solutions of the sodium salts of the parent and the new derivatives of ceftazidime were recorded on a double-beam UV-spectrophotometer and their λmax were determined. The λmax of ceftazidimepentahydrate is at 254nm, while λmax of ceftazidime Schiff base (compound 1) is at 299 nm. λmax of ceftazidime- lysine Schiff base (compound 2) is at 277nm. Under the experimental conditions [30], the behavior of the hydrolysis of compounds 1 and 2 follow pseudo-first order kinetic, since plot of log concentration vs. time resulted in a straight line and from the slope of this plot, the observed rate constant of hydrolysis was calculated. The degree of hydrolysis of compound 1(28µg/ml) in KCl/HCl buffer (0.2M,pH 1.2) and in phosphate buffer (0.2M, pH 7.4) incubated at 37 ℃ was studied at different time intervals (zero, 15, 30, 60,120 and 240 min).The half-life values were calculated from the pseudo-first order kinetic

law and found to be $t_{½}$ 3.48hrs and 18.78hrs respectively.

A similar procedure was conducted to study the stability of ceftazidime-lysine Schiff base (compound 2) in KCl/HCl buffer (0.2M,pH 1.2) and in phosphate buffer (0.2M, pH 7.4) and the $t_{½}$ values were calculated using the pseudo-first order kinetic law and the values equal to 3.13 hrs and 24hrs respectively. Accordingly, the above studies have indicated significant increase in the values of $t_{1/2}$ (18.78hrs) of compound 1 at slightly basic media (pH 7.4), which is much longer than the $t_{1/2}$ of ceftazidime (2hr)[31]. However, the stability of compound 1 in KCl/HCl buffer (0.2M, pH 1.2) showed that thet$_{1/2}$ was 3.48hrs compared with ceftazidime (2hr) [31].

Compound 2 has a $t_{1/2}$ value of 24hrs when incubated in phosphate buffer (0.2M, pH 7.4), which is a clear significant result that indicate a great stability of compound 2 at the slightly basic conditions (resembles the physiological pH). However, the stability of compound 2 in KCl/HCl buffer (0.2M, pH 1.2) was slightly better and reaching 3hrs, when compared with ceftazidime[31].

Table (1) Physical parameters and percent yield of the synthesizedcompounds

Compound	Physical appearance	Yield %	m.p. ^{o}C	Rf value
1	Brown to yellow powder	70	165 Decomposed	0.75 (A)
2a	White powder	50	195-197	0. 69 (B)
2	Pale brown powder	68	210 Decomposed	0.62 (B)
3	White to pale yellow powder	52	266 Decomposed	0.68 (B)
4	Brown powder	58	225 Decomposed	0.65 (A)
5	Pale yellow powder	50	284 Decomposed	0.62 (B)

Table (2) Elemental microanalysis of the synthesized compounds.

Compounds	Chemical formula	Elemental microanalysis %		
	Molecular weight	Element	Calculated	found
1	$C_{30}H_{26}N_6Na_2O_{10}S_2$ 740.09	C	48.65	48.57
		H	3.54	3.46
		N	11.35	11.24
		S	8.66	8.88
2	$C_{43}H_{42}N_8Na_2O_{11}S_2$ 956.95	C	53.97	53.36
		H	4.42	4.57
		N	11.71	11.84
		S	6.70	6.92
3	$C_{29}H_{34}N_8Na_2O_{11}S_2$ 780.74	C	44.61	44.79
		H	4.39	4.50
		N	14.35	14.10
		S	8.21	8.48
4	$C_{69}H_{74}N_{12}Na_2O_{13}S_2$ 1389.51	C	59.64	59.48
		H	5.37	5.57
		N	12.10	12.45
		S	4.62	4.38
5	$C_{41}H_{58}N_{12}Na_2O_{13}S_2$ 1037.08	C	47.48	47.59
		H	5.64	5.49
		N	16.21	16.55
		S	6.18	6.37

Antimicrobial Activity Assessment

The newly synthesized derivatives were tested for their antimicrobial activity by disc-diffusion method [32] against the following microorganisms:

(a) Gram-negative: *Escherichia coli* and *Pseudomonas aeruginosa*

(b) Gram-positive: *Streptococcus spp* and *Staphylococcusaureus*.

Type of media used: (Nutrient Medium), which contain 1g/L distilled water, peptone (5gm) and meat extract (3gm), and the pH was adjusted to 7.0. Each of the synthesized compounds (30 µg) was dissolved in dimethylsulphoxide to prepare the test solutions. Ceftazidime (30 µg) was used as the standard antibacterial drug. Dimethylsulphoxide: water mixture (1:30) was used as the solvent. The results are shown on Table (3).

Table (3) Antimicrobial activity evaluation of the synthesized compounds.

compounds	S. pyogen.	S. Aureus.	E. coli	P. aeruginosa
DMSO	-	-	-	-
Ceftazidime	-	+	++	++
1	+	+	++	++
2	++	++	++	++
3	-	+	+++	++
4	++	+	+++	+++
5	-	+	++	+
2a	+	-	+	-

Key to symbols: (-) = no inhibition, (14 mm) = +, (15-17 mm) = + + (more than 18 mm) = +++.

The antimicrobial screening revealed that the newly synthesized compounds (2 and 4) showed reasonable antibacterial activities against G (+) *Strep. Spp*. in comparison with ceftazidime, which has no activity against this type of microorganism. Compound 2 showed good activity against all 4 strains of bacteria used, as compared with ceftazidime. Compound 4 showed good and reasonable activities against *E. coli* and *P. aeruginosa* comparable with ceftazidime. While, it showed good activity against *Strep. Spp.* and moderate activity against *Staph. aureus*.

Compound 1 showed moderate activity against *Strep. Spp*. and staph Spp. and good activity against *E. coli* and *P. aeruginosa.* Compound 3 showed good and reasonable activity against *E. coli* and *P. aeruginosa* and moderately active towards *staph. Spp*. However, compound 3 showed no activity against *Strep. Spp*. Compound 5 showed good activity against *E. coli* and moderate activity against *P. aeruginosa*.

Generally, all the Schiff bases of ceftazidime (compounds 1, 2 and 4) showed good and reasonable antimicrobial activity against the tested microorganisms especially G (+) bacteria. This increase in activity may be due to the incorporation of extra imine groups.

The slight reduction in antibacterial activities of some of these derivatives (compounds 3 and 5) as compared with ceftazidime is observed, but these may still have potential to be used as therapeutic agents. Compounds 3 and 5 that devoid of the extra imine groups and contain two primary amine groups have moderate to slight activities as compared to ceftazidime. Compounds 2 and 4 showed broader antibacterial spectrum against both G (+) and G (-) bacteria.

The Schiff bases of lysine (2, 6-bis (benzylideneamino)) hexanoic acid (compound 2a) exhibit slight activities towards *E. coli* and *strep. Spp*, as compared with lysine which has no antibacterial activity. However, compound 2a showed no antimicrobial activities against S. aureus or *P. aeruginosa*.

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