Synthesis, Characterization, and Antimicrobial Evaluation of New Ceftriaxone Derivatives

Kasim S. Hmood[*] and Amer N. Elias[*, 1]

[*]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

The present study was designed to synthesize a number of new Ceftriaxone derivatives by its involvement with a series of different amines, through the chemical derivatization of its 2-aminothiazolyl- group into an amide with chloroacetyl chloride, which on further conjugation with these selected amines will produce compounds with pharmacological effects that may extend the antimicrobial activity of the parent compound depending on the nature of these moieties.

Ceftriaxone was first equipped with a spacer arm (linker) by the action of chloroacetyl chloride in aqueous medium and then further reacted with seven different aliphatic and aromatic amines which resulted in the production of the aimed final target products. The syntheses have been carried out following simple methodology in excellent isolated yields.

The structure of the synthesized derivatives has been characterized by elemental microanalysis (CHN), FTIR spectroscopy, and other physicochemical properties. All the final synthesized compounds were screened for their antimicrobial activity against selected microorganisms and showed excellent antibacterial and antifungal activities in comparison to Ceftriaxone, Cephalexin and Fluconazole.

The new Ceftriaxone derivatives have broadened the antimicrobial spectrum of the parent compound in having an extra antifungal activity in addition to its original antibacterial activity.

Keywords: Ceftriaxone, 2-aminothiazole, Antimicrobial activity, Antifungal activity, Ceftriaxone derivatives.

تصنيع وتوصيف وتقييم الفعالية المضادة للميكروبات لمشتقات جديدة للسيفترايكسون

قاسم سلمان حمود[*] و عامر ناظم الياس[*،1]

* فرع الكيمياء الصيدلانية ، كلية الصيدلة ،جامعة بغداد ، بغداد ، العراق .

الخلاصة

لقد صممت الدراسة الحالية لتصنيع عدد من مشتقات السيفترياكسون الجديدة و ذلك بمشاركته مع سلسلة من الامينات المختلفة من خلال تحضير الامايد من مجموعة ال 2-امينوثايزوليل- الخاصة به بواسطة كاشف كلوريد الكلوروأسيتيل والذي عند اقترانه بعد ذلك بتلك الامينات المختارة سيُنتج موادا لها تاثيرات فارماكولوجية قد ينجم عنها توسع في نشاط المركب الام ضد الميكروبات اعتمادا على طبيعة هذه المجاميع.

و لهذا فقد جهز السيفترياكسون اولا بذراع فاصل (رابط) بفعل مادة كلوريد الكلوروأسيتيل في محيط مائي و من ثم تمت مفاعلته بعدها بسبعة امينات اليفاتية و اروماتية مختلفة التي انتجت المواد النهائية المبتغاة . لقد اجري التصنيع بواسطة استخدام الطرق المنهجية البسيطة و بعوائد فصل ممتازة.

لقد مُيزَت التراكيب الكيمياوية لهذه المركبات باستخدام التحليل الدقيق للعناصر (CHN), اطياف الاشعة تحت الحمراء , و غيرها من الخواص الفيزيوكيمياوية . تم فحص فعالية كافة النواتج النهائية المضادة للميكروبات ضد عدد مختار من هذه الاحياء الدقيقة و التي اظهرت فعالية حسنة جدا ضد البكتيريا و الفطريات بالمقارنة مع السيفترياكسون, السيفاليكسين و الفلوكونازول.

أظهرت المشتقات الجديدة لعقار السيفترياكسون توسعاً في طيف المركب الام في فعاليته ضد الميكروبات من خلال امتلاك فعالية جديدة ضد الفطريات اضافةً الى نشاطه الاصلي ضد البكتيريا.

الكلمات المفتاحية : السيفترياكسون ، 2-امينوثايزول ، الفعالية ،الفعالية المضادة للميكروبات ، الفعالية المضادة للفطريات ، مشتقات السيفترايكسون .

Introduction

The irresponsible usage of drugs and among which the antimicrobial agents has affected the general population and specially the immunocompromised patients since it resulted in the corresponding increase of diseases caused by bacteria, fungi and viral species. Frankly speaking the widespread use of antibacterial and antifungal drugs has resulted in the increased resistance of the bacterial and fungal infections towards these drugs and has led to serious health hazards. The resistance that faced the wide spectrum antibacterial agents which increased dramatically in recent years has prompted the discovery and modification towards new antifungal and antibacterial drugs [1, 2], since the infections caused by these tough mutant microorganisms pose a serious challenge to the medical community and highlighted the importance and urgent need for new, more potent and selective non-traditional antimicrobial agents [3].

[1]Corresponding author E-mail: amerelias40@yahoo.com

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The rational planning of new synthetic prototypes in drug design and development has been based on the combination of pharmacophoric moieties of different bioactive substances [4] and using a series of methods of structural modification that aim, *a priori*, at the generation of new compounds presenting optimized pharmacodynamic and pharmacokinetic properties, exploring bioactive substances' fragments (Fragment-Based Drug Design) [5], active metabolites of drugs [6], bioisosterism [7, 8], selective optimization of side effects of drugs [9] and drug latentiation [10].

The drug latentiation strategy, in principle, uses a functional group in a drug as merely a handle for the introduction of a moiety that confers on the new entity some desirable characteristic to improve its pharmaceutical utility; more frequently, the group is intimately connected with the pharmaceutical deficiency and its masking directly addresses the deficiency. Of the commonly occurring drug functional groups, perhaps greatest effort has been directed at temporarily masking the amino group. The most easily identified liability of candidate amino drugs is their tendency to ionize under physiological conditions, leading to poor membrane penetration by passive diffusion. The impact of this is amplified for the large number of amino drugs that are required to penetrate the blood brain barrier in order to reach their pharmacological targets. A second issue that can affect the development of amino drugs is instability. An example of this is the tendency of primary amines to undergo first-pass metabolism due to N-acetylation and oxidation by monoaminooxidase (MAO) [11]. Low water solubility, poor stability and low permeability through biological membranes often hinder the clinical development of biologically active amino compounds [12, 13]. The major advantage in designing derivatives of amino drugs is the general robustness of amine derivatives particularly those, such as amides, in which the capacity to ionize has been obviated [14, 15]. On the other hand, the very robustness of amino derivatives means that subtle drug targeting effects can be achieved if an appropriate local vector can be identified and accommodated in the design process [16].

Derivatives of amino compounds such as piperazine, 2-aminothiazole and others were reported earlier in the literature in that they have an extended and diverse biological activity when compared to their parent compounds [17-28]. Sometimes, they may even attain a complete change in their biological responses as illustrated for the N-alkyl and N-aryl derivatives of piperazine in that they possess antibacterial and antifungal activities which are even not present in their parent compound, the well-known anthelmintic piperazine [17, 18, 20, 21, 26, and 27].

Thiazole derivatives have been found to possess many pharmacological properties and are widely implicated in biochemical processes. The 2-Aminothiazole can be used as a thyroid inhibitor in the treatment of hyperthyroidism or for its antibacterial activity [29, 30]. Recent studies using prion-infected neuroblastoma cell lines have suggested that the 2-aminothiazole may be used as a therapeutic drug for prion diseases [31]. Members of this class of thiazoles are known to possess antibacterial activity against both gram-positive and gram-negative bacteria, for e.g. Ceftriaxone, a semi-synthetic third generation cephalosporin that even shows excellent activity for the treatment of infection due to methicillin-susceptible *staphylococcus aureus* [32].

Ceftriaxone is marketed for parenteral use with a relatively long half-life and it is stable to the β-lactamases particularly those produced by Gram-negative organisms [33, 34], which is due to the fact it is a competitive, irreversible beta-lactamase inhibitor and has good inhibitory activities against the clinically important plasmid mediated β-lactamase that is most frequently responsible for the transferred drug resistance [33, 34]. It has excellent anti Gram-negative activity. It contains a highly acidic heterocyclic system on the 3-thiomethyl group; where this unusual ring system is believed to confer the unique pharmacokinetic properties to this agent. It kills bacteria by interfering in the synthesis of the cell wall. Ceftriaxone has been effective in treating infections due to other 'difficult' organisms such as the multidrug-resistant Enterobacteriaceae [35].

So far, the modifications of the thiazole ring have proven to be highly effective with improved potency and lesser toxicity [30]. In continuation of the increased interest in the chemistry of functionalized chloroacetamide derivatives in drug discovery, because of the high mobility of chlorine atom and the reactive N-H group, therefore, compounds containing chloroacetamide moiety are known to be useful synthetic scaffolds for the design of many heterocyclic systems [36, 37]. In this context Patten and coworkers have reviewed the synthesis of a variety of the 2-aminothiazoles and their substituted derivatives (by first introducing the α-chloroamides of this amine and later by conjugating it with other amino compounds

such as piperidine, morpholine and others), and evaluating the antibacterial and antifungal activities of these synthesized products [38]. It was observed that the substituted thiazoles have very good antibacterial and antifungal activities against all the tested samples whereas the chemotherapeutic agents used were active against some specific samples [38].

Therefore, the present study was designed to synthesize a number of new Ceftriaxone derivatives; by first converting its 2-aminothiazolyl- moiety into the α-chloroamide derivative which on further conjugation with selected amines will result in the production of the final compounds; that may possibly broaden the antimicrobial spectrum of their parent Ceftriaxone.

Materials and Methods

Materials and Equipments

2-Aminothiazole and Potassium carbonate were purchased from Himedia (India), Chloroacetyl chloride and Imidazole were purchased from Fluka AG (Switzerland), Morpholine was purchased from Lobachemie (India), Piperidine was purchased from BDH (England), Hydrazine hydrate (80% w/v) was purchased from Qualikems (India), and Pyrrolidine and N-methylpiprazine were purchased from sigma (Germany).

Ceftriaxone sodium was donated thankfully by the Arab Company for Antibiotics Industries (ACAI) (Baghdad, Iraq). The quality of all these chemicals together with the other ones used throughout the study and obtained from standard commercial sources were of analar grade and used without further purification.

The melting points were determined by the open capillary method using Thomas hoover melting point apparatus (England) and were used uncorrected. Cooling of reactions when needed was done using a Julabo chiller VC (F30) (GMBH, Germany). Infra-red spectra were recorded in KBr disc on Shimadzu FTIR 8400 spectrometer (Japan), at the College of Pharmacy, University of Baghdad and the College of Science, University of Al-Mustansiriyah. Elemental microanalysis was performed at the Jordanian University using CHN Elemental Analyzer (Euro-vector EA3000A, Italy) and at the Department of Chemistry, College of Science, Al-Mustansiriyah University, using CHNS Elemental Analyzer (Euro-vector EA, Italy).

The progress of the reaction was monitored by ascending thin layer chromatography which was run on Kieslgel GF_{254} (60) aluminum plates (E. Merck, Germany), which was used as well to check the purity of the product. The synthesized compound was revealed either by derivatization or reactivity toward iodine vapor or by irradiation with UV_{254} light. Chromatograms were eluted using methanol: acetone: water (2:2:1) solvent system.

Chemical tests such as the sodium fusion and the carboxylic acid tests were run to check the presence or absence of chlorine [39] and the free carboxylic acid group [40] in all the synthesized compounds.

The antimicrobial evaluation was performed at the Department of biology, College of Science, University of Baghdad.

Experimental Section

A. Chemical synthesis

1. Synthesis of (6R,7R)-7-((Z)-2-(2-(2-chloroacetamido)thiazol-4-yl)-2-(methoxyimino)acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Intermediate compound A)

Intermediate (A) was synthesized according to the synthetic pathway depicted in Scheme 1 and in accordance to the previously reported procedure in reference 41 for the synthesis of α-chloroamides in water.

An accurately weighed amount of ceftriaxone disodium (10 mmol, 6.62 gm) dissolved in 20 ml of distilled water was placed in a 100ml round bottom flask. Triethylamine (Et_3N or TEA) (10 mmol, 1.4 ml) was added to this solution and the mixture was cooled in an ice salt bath at (-10^o C). Chloroacetyl chloride (40 mmol, 3.2 ml) was added drop wise to the above mentioned mixture with constant stirring over a period of one hour, while keeping the temperature at (-10^o C). The mixture was left to stir overnight and the product was isolated as a faint yellowish white precipitate. The pH of the supernatant liquid was measured and it was found to be strongly acidic around 3.5-4.0. The precipitated material was washed three times with 50 ml of distilled water, and then left to dry. This derivative was recrystallized from acetone: methanol (1:1).

The percent yield, physical appearance, melting point, and R_f value of the synthesized intermediate compound are listed in Table 1, while its elemental microanalysis and the FTIR spectral data assignments of bands are shown in Tables 2 and 3 respectively.

Scheme (1): The synthetic scheme of the intermediate compound (A)

2. The chemical synthesis of the final Ceftriaxone derivatives (Target compounds B1-B7) [42].

To a stirred mixture of the intermediate compound (A) (5 mmol, 1.57 gm) and K_2CO_3 (10 mmol, 0.98 gm) in 50 ml of acetone, was added one of the following amines (5 mmol): Morpholine (0.48 ml), Pyrrolidine (0.42 ml), Piperidine (0.44 ml), Hydrazine hydrate (0.5 ml), N-methylpiprazine (0.54 ml), Imidazole (0.335 gm), and 2-Aminothiazole (0.5 gm) and then the resultant mixture was refluxed for 16-18 hours at 60-70^o C. Later the solvent was evaporated under reduced pressure, and the precipitate obtained was collected and washed several times (3X) with 5 ml portions of acetone. The solid obtained after washing showed only one TLC spot which corresponds originally to the intended product appeared during the reaction follow-up. Later the obtained product was recrystallized from aqueous ethanol. The synthetic scheme of the final Ceftriaxone derivatives (target compounds B1-B7) is shown in scheme 2.

The percent yield, physical appearance, melting points, and R_f values of the synthesized compounds are listed in Table 1, while their elemental microanalysis and the FTIR spectral data assignments of bands are shown in Tables 2 and 3 respectively.

Scheme(2): The synthetic scheme of the final Ceftriaxone derivatives (target compounds B1-B7)

Table(1): The percent yield, physical appearance, uncorrected Melting points, and R_f values of the synthesized intermediate (A) and target compounds (B1-B7)

Compound	*Chemical Name*	*Chemical Formula*	*Physical appearance*	*% Yield*	*Melting point ^{O}C*	*R_f*
A	(6R,7R)-7-((Z)-2-(2-(2-chloroacetamido)thiazol-4-yl)-2-(methoxyimino)-acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{20}H_{19}ClN_8O_8S_3$	White powder	65	172-174	0.26
B1	(6R,7R)-7-((Z)-2-(methoxyimino)-2-(2-(2-morpholinoacetamido)-thiazol-4-yl)acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{24}H_{27}N_9O_9S_3$	yellow powder	88	245	0.5
B2	(6R,7R)-7-((Z)-2-(methoxyimino)-2-(2-(2-(pyrrolidin-1-yl)-acetamido)thiazol-4-yl)acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{24}H_{27}N_9O_8S_3$	brown powder	93	230	0.55
B3	(6R,7R)-7-((Z)-2-(methoxyimino)-2-(2-(2-(piperidin-1-yl)-acetamido)thiazol-4-yl)acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{25}H_{29}N_9O_8S_3$	pale yellow powder	88	220	0.64
B4	(6R,7R)-7-((Z)-2-(2-(2-hydrazinylacetamido)-thiazol-4-yl)-2-(methoxyimino)-acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{20}H_{22}N_{10}O_8S_3$	Dark yellow powder	87	220	0.66
B5	(6R,7R)-7-((Z)-2-(methoxyimino)-2-(2-(2-(4-methylpiperazin-1-yl)acetamido)thiazol-4-yl)acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{25}H_{30}N_{10}O_8S_3$	pale brown powder	86	247-249	0.68

B6	(6R,7R)-7-((Z)-2-(2-(2-(1H-imidazol-1-yl)acetamido)thiazol-4-yl)-2-(methoxyimino)-acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{23}H_{22}N_{10}O_8S_3$	Light brown powder	90	231-233	0.56
B7	(6R,7R)-7-((Z)-2-(methoxyimino)-2-(2-(2-(thiazol-2-ylamino)-acetamido)thiazol-4-yl)acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	$C_{23}H_{22}N_{10}O_8S_4$	Yellow powder	89	184-186	0.58

Table (2): The elemental microanalysis (CHNS) of the synthesized intermediate (A) and target compounds (B1-B7)

Compound	*Chemical Formula*	*Mol. wt.*	*Value type*	*C*	*H*	*N*	*S*
A	$C_{20}H_{19}ClN_8O_8S_3$	630.02	calculated observed	38.07 38.319	3.03 3.042	17.76 17.791	15.24 15.312
B1	$C_{24}H_{27}N_9O_9S_3$	681.72	calculated observed	42.28 41.958	3.99 3.898	18.49 18.267	14.11 14.551
B2	$C_{24}H_{27}N_9O_8S_3$	665.72	calculated observed	43.3 44.147	4.09 4.12	18.94 19.22	14.45 14.5
B3	$C_{25}H_{29}N_9O_8S_3$	679.75	calculated observed	44.17 44.674	4.3 4.360	18.55 18.393	*
B4	$C_{20}H_{22}N_{10}O_8S_3$	626.65	calculated observed	38.33 38.198	3.54 3.444	22.35 22.103	*
B5	$C_{25}H_{30}N_{10}O_8S_3$	694.76	calculated observed	43.22 43.352	4.35 4.411	20.16 19.985	*
B6	$C_{23}H_{22}N_{10}O_8S_3$	662.68	calculated observed	41.69 40.923	3.35 3.282	21.14 21.938	14.52 14.274
B7	$C_{23}H_{22}N_{10}O_8S_4$	694.74	calculated observed	39.76 40.145	3.19 3.22	20.16 20.704	18.46 18.314

* The analysis was done at the Jordanian University which lacks the detection of sulfur.

Table (3): The characteristic IR bands of the synthesized intermediate (A) and target compounds (B1-B7).

Sym.	*Chemical structure*	*IR band [KBr] ν cm^{-1}*	*Interpretation*
A		3379	(N-H) stretching vibration of secondary amide
		3103	heteroaromatic (C-H) stretching vibration
		2940	aliphatic (C-H) asymmetric stretching vibration
		2840	aliphatic (C-H) symmetric stretching vibration
		1774	(C=O) Stretching vibration of β-lactam
		1713	(C=O) stretching vibration of –COOH group
		1658	(C=O) stretching vibration of secondary amide
		1631	Oxime C=N stretching mode
		1586	Stretching vibration of (C=C) in the Thiazole ring
		1448	(C-H) bending vibration of $–CH_2$ group
		1413	(C-H) bending vibration of $–CH_3$ group
		1311	(C-O) stretching of –COOH group
		1246, 1222	(C-H) wag in terminal $-CH_2Cl$
		1184	(C-O) stretching vibration
		1097, 1045	(C-O) stretching in $-OCH_3$ and (N-O) in oxime
		852	(C-Cl) stretching vibration
B1		3439	(N-H) stretching vibration of secondary amide
		2980	aliphatic (C-H) asymmetric stretching vibration
		2890	aliphatic (C-H) symmetric stretching vibration
		1761	(C=O) Stretching vibration of β-lactam
		1712	(C=O) stretching vibration of –COOH group
		1643	(C=O) stretching vibration of secondary amide
		1608	Oxime C=N stretching mode
		1595	Stretching vibration of (C=C) in the Thiazole ring
		1460	(C-H) bending vibration of $–CH_2$ group
		1375	(C-H) in-plane bending vibration of $-CH_2$ group
		1300	(C-O) stretching of –COOH group
		1215	(C-N) stretching of amino group
		1184	(C-O) stretching vibration
		1107, 1039	(C-O) and (N-O) stretching in $-OCH_3$ and oxime
		808	heteroaromatic (C-H) out of plane bending
B2		3375	(N-H) stretching vibration of secondary amide
		2941	aliphatic (C-H) asymmetric stretching vibration
		2854	aliphatic (C-H) symmetric stretching vibration
		1769	(C=O) Stretching vibration of β-lactam
		1716	(C=O) stretching vibration of –COOH group
		1670	(C=O) stretching vibration of secondary amide
		1637	Oxime C=N stretching mode
		1586	Stretching vibration of (C=C) in the Thiazole ring
		1460	(C-H) bending vibration of $–CH_2$ group
		1369	(C-H) bending vibration of $–CH_3$ group
		1261	(C-O) stretching of –COOH group
		1220	(C-N) stretching of amino group
		1070, 1041	(C-O) and (N-O) stretching in $-OCH_3$ and oxime

		833	heteroaromatic (C-H) out-of-plane bending
B3		3400	(N-H) stretching vibration of secondary amide
		2941	aliphatic (C-H) asymmetric stretching vibration
		2840	aliphatic (C-H) symmetric stretching vibration
		1769	(C=O) Stretching vibration of β-lactam
		1712	(C=O) stretching vibration of –COOH group (shoulder)
		1654	(C=O) stretching vibration of secondary amide
		1604	Oxime C=N stretching mode
		1537	Stretching vibration of (C=C) in the Thiazole ring
		1460	(C-H) bending vibration of $–CH_2$ group
		1367	(C-H) in-plane bending vibration of $–CH_3$ group
		1292	(C-O) stretching of –COOH group
		1215	(C-N) stretching of amino group
		1101, 1039	(C-O) and (N-O) stretching in $-OCH_3$ and oxime
		823	heteroaromatic (C-H) out-of-plane bending
B4		3462-3311	(N-H) stretching vibrations of primary –NH2 group around 3462 and 3400 cm^{-1} coupled with the band between 3350-3310 cm^{-1} for the secondary amine of the reacted hydrazine
		3425	(N-H) stretching vibration of secondary amide
		2945	aliphatic (C-H) asymmetric stretching vibration
		2825	aliphatic (C-H) symmetric stretching vibration
		1759	(C=O) Stretching vibration of β-lactam
		1710	(C=O) stretching vibration of –COOH group (shoulder)
		1635	(C=O) stretching vibration of secondary amide
		1604	Oxime C=N stretching mode (shoulder)
		1550	Stretching vibration of (C=C) in the Thiazole ring
		1525	(N-H) bending vibration of secondary amide
		1456	(C-H) bending vibration of $–CH_2$ group
		1375	(C-H) bending vibration of $–CH_3$ group
		1272	(C-O) stretching of –COOH group
		1207	(C-N) stretching of amino group
		1134, 1041	(C-O) and (N-O) stretching in $-OCH_3$ and oxime
		806	heteroaromatic (C-H) out-of-plane bending
B5		3419	(N-H) stretching vibration of secondary amide
		2945	aliphatic (C-H) asymmetric stretching vibration
		2823	aliphatic (C-H) symmetric stretching vibration
		1761	(C=O) Stretching vibration of β-lactam
		1710	(C=O) stretching vibration of –COOH group (shoulder)
		1651	(C=O) stretching vibration of secondary amide
		1604	Oxime C=N stretching mode (shoulder)
		1558	Stretching vibration of (C=C) in the Thiazole ring
		1440	(C-H) bending vibration of $–CH_2$ group
		1371	(C-H) in-plane bending vibration of $–CH_3$ group
		1290	(C-O) stretching of –COOH group
		1215	(C-N) stretching of amino group
		1107, 1037	(C-O) and (N-O) stretching in $-OCH_3$ and oxime
		833	heteroaromatic (C-H) out-of-plane bending
		3394	(N-H) stretching vibration of secondary amide
		2943	aliphatic (C-H) asymmetric stretching vibration

B6		2872	aliphatic (C-H) symmetric stretching vibration
		1751	(C=O) Stretching vibration of β-lactam
		1715	(C=O) stretching vibration of –COOH group
		1664	(C=O) stretching vibration of secondary amide
		1650	(C=O) stretching vibration of amide
		1606	Oxime C=N stretching mode
		1535	Stretching vibration of (C=C) in the Thiazole ring
		1440	(C-H) bending vibration of $–CH_2$ group (shoulder)
		1375	(C-H) in-plane bending vibration of $–CH_3$ group
		1292	(C-O) stretching of –COOH group
		1215	(C-N) stretching of amino group
		1109, 1041	(C-O) and (N-O) stretching in $-OCH_3$ and oxime
		833	heteroaromatic (C-H) out-of-plane bending
B7		3454	(N-H) stretching vibration of secondary amide
		3342	(N-H) stretching vibration of secondary amine
		2943	aliphatic (C-H) asymmetric stretching vibration
		2823	aliphatic (C-H) symmetric stretching vibration
		1751	(C=O) Stretching vibration of β-lactam
		1720	(C=O) stretching vibration of –COOH group
		1653	(C=O) stretching vibration of secondary amide
		1616	Oxime C=N stretching mode
		1550	Stretching vibration of (C=C) in the Thiazole ring
		1464	(C-H) bending vibration of $–CH_2$ group
		1377	(C-H) in-plane bending vibration of $–CH_3$ group
		1276	(C-O) stretching of –COOH group
		1207	(C-N) stretching of amino group
		1103, 1041	(C-O) and (N-O) stretching in $-OCH_3$ and oxime
		856	heteroaromatic (C-H) out-of-plane bending

Notes: 1. It was noticed that the (C=O) stretching vibration of the newly formed amide in the intermediate (A) and the final compounds (B1-B7) and that of the other amide already present in Ceftriaxone were overlapping each other.

2. It is clear that the (C-Cl) stretching vibration appeared in the intermediate (A) at 852 cm^{-1} has disappeared in the final target compounds (B1-B7).

B. The antimicrobial evaluation of the newly synthesized ceftriaxone derivative

The antimicrobial activity was determined for the newly synthesized Ceftriaxone derivatives by the well diffusion method for screening the *in vitro* antibacterial activity against the selected Gram positive (*Staphylococcus aureus)* and Gram negative bacteria (*E. coli*) and screening the antifungal activity against the selected fungus (*Candida albican*s) [43]. Ceftriaxone, Cephalexin were used as references for testing the antibacterial activity while Fluconazole was used as a reference for testing the antifungal activity. The synthesized compounds and references were dissolved in DMSO (also was used as a control), to prepare a 500 μg/ml stock solution. Two dilutions of the synthesized compounds and reference compounds were prepared to have 250 and 125 μg/ml respectively [44]. Wells were made in Mueller Hinton agar for bacteria and sabouraud dextrose agar for *C. albicans*. Plates were seeded with 0.1 ml of 10^8 CFU / ml of bacteria, and with 10^6 CFU / ml of *C. albicans*. Triplicates of each concentration for each microorganism species were prepared. The inoculated plates were incubated at 37° C for 24 hr. The diameter of the inhibition zones were measured for each plate [45]. The inhibitory zones for each of the synthesized and reference compounds against each of the tested microorganisms are listed in Tables 4 and 5.

Table (4): The antibacterial activity data of the synthesized target compounds

Compound	*Zone of inhibition in mm*			
	Staphylococcus aureus		*E. coli*	
	125 µg/ml	*250 µg/ml*	*125 µg/ml*	*250 µg/ml*
B1	26	32	22	29
B2	18	18	15	17
B3	31	35	27	30
B4	23	27	22	25
B5	27	30	29	30
B6	26	27	22	29
B7	26	27	27	28
DMSO	-	-	-	-
Fluconazole	-	-	-	-
Cephalexin	30	38	33	37
Ceftriaxone	32	34	32	33

Table (5): The antifungal activity data of the synthesized target compounds

Compound	*Zone of inhibition in mm*	
	C. albicans	
	125 µg/ml	*250 µg/ml*
B1	26	27
B2	16	16
B3	30	32
B4	25	28
B5	25	31
B6	25	26
B7	22	27
DMSO	-	-
Fluconazole	33	37
Cephalexin	-	-
Ceftriaxone	-	-

Notes: 1. DMSO was used as a control for both the antibacterial and antifungal evaluations and it did not show any inhibitory activity against both the bacteria and fungus used in these tests.
2. Running Ceftriaxone and Cephalexin in the antimicrobial tests showed that these two chemotherapeutic agents didn't have any antifungal activity originally, while Fluconazole on the other hand showed that it lacks any antibacterial activity.

Results and Discussion

The scope of the research includes first of all equipping Ceftriaxone with the desired spacer moiety by acylating its 2-aminothiazolyl- group with chloroacetyl chloride which subsequently forming its α-chloroamide derivative (intermediate A). Secondly the latter was further conjugated with several amines in order to produce the final target compounds (B1-B7).

The strategy that lies behind the synthesis of this system is in the fact that the chemical synthesis of amides are conducted in organic solutions or in a mixture of organic and aqueous solutions (Schotten–Baumann conditions) where the organic reagents are generally dissolved in the organic solution and treated with aqueous base [46].

The development of alternative methods for achieving amide synthesis in high yield and/or in a stereospecific manner is of great current interest. It was reported earlier that the classical reaction between the corresponding amine and chloroacetyl chloride (2 equiv.) in dry tetrahydrofuran (or dichloromethane) at -10 ^{o}C in the presence of freshly distilled triethylamine, gave very low yields of the desired α-chloroamides. Furthermore, changing to solvents such as

dimethylformamide, dichloromethane, chloroform or diethyl ether did not increase significantly the yield of the products, and the reaction only proceeded in ethyl acetate, but in low yields [47].

Hence, the focus was directed towards alternative synthetic methods and it was found that the amide coupling could be conducted in water in the presence of a medium of bases such as Et_3N, $KHCO_3$, K_2CO_3 or NaOH, where the desired products were formed as solids in acceptable yields, under ambient conditions [41].

Varying the reaction conditions such as solvent and base did not yield any improvement. The problem seemed to stem from the insolubility of the amine, which was only fully soluble in alcohols and water [48].

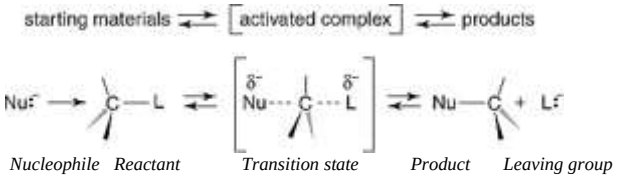
However, due to the lack of solubility of the amine in the organic solution; it was decided to carry out a modification of this method using only water as the solvent. Moreover, instead of using an excess of the amine, four equiv. of chloroacetyl chloride were used, which were added dropwise over 1 h to the aqueous amine solution. The solution was left to stir overnight and the desired product was isolated as a precipitate in yields of 65% with no need for further purification.

Hence, the two-phase procedure mentioned earlier was not necessary and in fact the modification which was carried out here enabled the successful isolation of the desired product with minimal effort.

Accordingly, the free 2-aminothiazolyl-group of Ceftriaxone sodium was found to react in water with the spacer compound (chloroacetyl chloride) in the presence of a suitable base such as Et_3N and this will result in the formation of the intended corresponding α-chloroamide [49].

The mechanism of this reaction is an acyl nucleophilic substitution which occurs selectively at the acyl carbon atom in chloroacetyl chloride because of the greater reactivity of nucleophiles toward acid chlorides compared to alkyl chlorides. The reasons for this selectivity are attributed to the differences in the electrophilicity of the two carbon atoms in chloroacetyl chloride. Besides that the electronic, and steric factors also play a role in this selectivity, since it is easier for the nucleophile to attack the carbon of the planar carbonyl group in the acid chloride than to attack the tetrahedral carbon in the $–CH_2Cl$ group. The reaction is carried out with triethylamine, which acts as a base to neutralize the hydrogen chloride (HCl) formed [50, 51].

Later the selected amines are coupled with the synthesized intermediate compound (A) for the synthesis of the target compounds (B1-B7), since the amine **N** functions as a nucleophile and attacks the electrophilic **C** of the alkyl chloride displacing the chloride and creating the new **C-N** bond via an alkyl nucleophilic substitution reaction (S_N2), to yield the corresponding amine alkylated product. The S_N2 mechanism is concerted and proceeds through a single rate-determining transition step and it begins when the reactant is attacked by a nucleophile from the side opposite the leaving group, with bond making occurring simultaneously with bond breaking between the carbon atom and the leaving group. The transition state has trigonal bipyramidal geometry shape with a pentacoordinated carbon. With the loss of the leaving group, the carbon atom again assumes a pyramidal shape; however, its configuration is inverted and this inversion is often called the Walden inversion as shown in Scheme 3 [52-55].



(Note: The product presents the Walden inversion in being Stereochemically inverted)

Scheme(3): The mechanism of the S_N2 reaction

The structures of the synthesized compounds were confirmed by using FTIR, elemental microanalysis (CHNS), and other physicochemical parameters (Tables 1, 2, and 3). The FTIR spectrum of Ceftriaxone sodium obtained in KBr pellets was in excellent agreement with that reported earlier in the literature [56]. The synthesized compounds (A and B1-B7) showed several characteristic sharp bands in the IR region, where the appearance of the band near 3500-3400 cm^{-1} that represent the free NH stretching vibration of the formed secondary amide was accompanied with the disappearance of the two peaks near 3500 and 3400 cm^{-1} of the N-H stretching modes of primary amine of the 2-aminothiazolyl- moiety, and this was accompanied together with the appearance of the signal near 1640 cm^{-1} that represent the C=O stretching frequency of the carbonyl group in simple, open chain, secondary amides. It is also noticed that the (C-Cl) stretching vibration appeared in the intermediate (**A**) at 852 cm^{-1} has disappeared in all the final target compounds (B1-B7). The appearance of the signal in the region of 1720-1706 cm^{-1} represents the carbonyl group

stretching vibration of bonded aliphatic acids which is accompanied also with the appearance of very broad, intense O-H stretching absorption in the region of 3300-2500 cm^{-1} that corresponds also to carboxylic acid dimers. The two stretching vibrations that represent the carboxylate anion present previously in Ceftriaxone sodium at 1602 and 1398 cm^{-1} have disappeared from the spectrums of all synthesized compounds. It is worthwhile to mention that the chemical tests performed on all the synthesized compounds showed the appearance of chlorine in the intermediate compound (A) and its disappearance in the corresponding final compounds, while the carboxylic acid test showed the presence of the free carboxylic acid group in the synthesized intermediate and final derivatives.

The elemental microanalysis (Table 2) revealed good agreement with the calculated percentages.

The newly synthesized Ceftriaxone derivatives showed moderate to good antibacterial and antifungal activities. Both compounds B3 and B5 showed good activity compared to both Cephalexin and Ceftriaxone but compound B3 was more potent than Ceftriaxone in its antibacterial activity against *Staphylococcus aureus*. In addition all compounds showed moderate to good antifungal activity but compounds B3 and B5 were more potent than others.

The antimicrobial data presented earlier showed that the synthesized target compounds with the structural changes done with the parent Ceftriaxone molecule have gained an expansion in their antimicrobial activity to include antifungal properties in addition to the already retained antibacterial ones. The data showed also that Ceftriaxone itself lacks any activity against fungi in its antimicrobial spectrum.

Conclusion

The results obtained in the present study showed that it is possible to synthesize new derivatives of Ceftriaxone by linking its molecule through a spacer to appropriately chosen amines, since these amines may give by their presence additional properties to the Ceftriaxone parent compound. This was evidenced since the synthesized Ceftriaxone derivatives showed marked antibacterial and antifungal activities when compared with Ceftriaxone itself, Cephalexin and with Fluconazole. Therefore, these results illustrate that these Ceftriaxone derivatives have gained an expansion in their antimicrobial spectrum to include an added antifungal activity to it, while retaining the original antibacterial activity shown by the parent compound.

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