Synthesis of New Cephalosporins of Expected Improved Activity and Resistance Against β-Lactamases

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Abstract

The development of new cephalosporins with improved activity against resistant microbes, such as, MRSA (methicillin resistant *Staph. aureus*), *P. aeruginosa*, is of high potential. Chemical synthesis of two new series of thiadiazole linked to cysteine (series 1) and cephalosporins containing thiadiazole linked to cysteine through disulfide bond (series 2) were achieved. The chemical structures of the synthesized compounds were confirmed using spectral (FT-IR, ^{1}H-NMR) and elemental microanalysis. The incorporation of privileged chemical moieties, such as, thiadiazole, Schiff base, cysteine and sulfonamide, has been found to have great contribution to the antimicrobial activities. Compounds of series 1 (**1b-d**), containing a Schiff base or a sulfonamido moiety, showed reasonable activity and were less potent than cephalexin with respect to *E. coli* and *Staph. aureus*. The new cephalosporins (series 2) showed remarkable activities on *E. coli* (62.5-15.6µg/ml) and *staph. aureus* (31.2-62.5µg/ml) when compared with cephalexin (250 and 125 µg/ml) respectively. Moreover, compounds **1** and **3** showed very promising activity against MRSA (250 and 500µg/ml) respectively. The incorporation of a sulfonamido moiety to the cephalosporin molecule was successfully achieved. This is a very interesting finding which may open a new approach in the synthesis of newer cephalosporins.

Keywords: Cephalosporins, Cysteine, Schiff base, Sulfonamides, Thiadiazole.

تخليق وتشخيص وتقييم اولي للفعالية المضادة للبكتريا لمشتقات جديدة لمجموعة 1,3,4- ثايا دايازول المرتبطة مع السيفالوسبورينات

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الخلاصة

ان تطوير سيفالوسبورينات جديدة ذات فعالية ضد المايكروبات المقاومة مثل المكورات العنقودية الذهبية المقاومة للمثيسيلين (MRSA)و الزائفة الزنجارية (*P. aeruginosa*) ذو اهمية عالية. لقد تم التخليق الكيميائي لاثنين من السلاسل الجديدة للثيادايزول المتصل بالحامض الاميني السيستاين (سلسلة 1) و سيفالوسبورينات الحاوية على الثيادايازول المتصل بالسيستاين بواسطة اصرة الدايسلفايد (سلسلة 2) . تم تشخيص التراكيب الكيميائية للمركبات المحضرة باستعما ل التحاليل الطيفية (تحويل فورييه مطياف الأشعة تحت الحمراء والرنين النووي المغناطيسي للبروتون) وقياس طيف الكتلة (الكاربون, الهايدروجين والنايتروجين). ان ادخال مجاميع كيميائية متميزة مثل الثيادايازول , قاعدة شف , سيستاين ومجموعة السلفون اميدو , وجد لها مساهمة ع الية في الفعاليات المضاده للمايكروبات. مركبات السلسلة الاولى **(1 a-d)** شف الحاوية على مجاميع قواعد شف او السلفون اميدو , اظهرت فعالية مقبولة وكانت اقل قوة من السيفاليكسين ضد البكتريا الاشريكية القولونية (*E. coli*) و فعاليات استثنائية ضد الزائفة الزنجارية . السيفالوسبورينات الجديدة (سلسلة 2) (15.6-62.5 مايكروغم/مل) و المكورات العنقودية الذهبية (31.2-62.5 مايكروغم/مل) اذا ما قورنت مع السيفاليكسين (250 و 125 مايكروغم/مل) على التوالي. بالاضافة الى المركبات **1** و**3** اظهرت فعالية واعده جدا ضد المكورات العنقودية الذهبية المقاومة للمثيسيلين (250 و 500 مايكروغم/مل) على التوالي. لقد تم بنجاح اضافة مجموعة السلفون اميدو الى السلسلة الجانبية على C7 لجزيئة السيفالوسبورين . ان التوصل الى هذه النتيجة المشجعة جدا يمكن ان يفتح افاق جديدة في تخليق سيفالوسبورينات جديدة تحوي هذه الصيغة الكيمياوية.

الكلمات المفتاحية : السيفالوسبورينات ، السيستاين ، قاعدة شف، السلفوناميد،الثيادايازول .

Introduction

The development of new antibiotics has been a very important task in providing the proper means for treating resistant strains of organisms that previously had been susceptible to older antibiotic [(1)]. The elucidation of biochemical mechanisms of microbial resistance to antibiotics, such as the inactivation of penicillins and cephalosporins by β-lactamases, has stimulated the research in the development of semisynthetic analogs that resist microbial biotransformation [(2)]. The evolution of hospital-acquired strains of staphylococci resistant to penicillin and of G (-) bacilli, such as, *Pseudomonas* and *Klebsiella spp*., *E. coli*, and others, often resistant to several antibiotics has become a serious medical problem[(3)].

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Received: 27/1/2014

Accepted:11 /6/2014

Cephalosporins are derived from cephalosporanic acid substituted at C_7 acyl side chain and at C_3 positions of the cephem. These derivatives may have different antibacterial spectra, β-lactamase sensitivity/resistance and pharmacokinetic properties [4]. The newer generations of cephalosporins have generally focused on two parameters: broadening the spectrum to include activity towards resistant pathogens and have anti-pseudomonal activity, as well as improving the pharmacokinetic properties [3, 5]. Cephalosporins linked to privileged chemical moieties, such as amino acids act as false substrate to the enzymes involved in bacterial cell wall synthesis [6]. Prodrugs of ceftizoxime containing an amino acid on the aminothiazol moiety was synthesized and found with potential activity and improved physicochemical properties and oral absorption [7]. Cephalosporins containing acyl derivatives of certain amino acids at the C_3 side chain showed improved aqueous solubility at physiological pH [8]. Thiadiazole ring was previously incorporated at C3 and C7 positions of the cephem, as privileged heterocyclic moiety. Cephalosporins containing thiadiazole showed good antibacterial activity against both G (+) and G (-) bacteria, such as, Cefuzonam, cephazoline, ceftobiprole and ceftaroline [9, 10]. Cefuzonam exhibited excellent activity against *P. aeruginosa* [11]. Cefazopran derivatives had anti-methicillin resistant *Staph. aureus* (MRSA) activity [12, 13]. Ceftobiprole and Ceftaroline fosamil are fifth generation cephalosporins containing thiadiazole moiety at C_7 position with activity against MRSA, penicillin-resistant *Strep. pneumonia*, *P. aeruginosa* and *Enterococci* [14,15]. Cephalosporins containing a 1,3,4-thiadiazole moiety linked through a sulfide or a disulfide bond in the acyl side chain were found to be equipotent to cephalexin [10]. This finding was supported when compared with antibiotics containing disulfide bonds and showed marked activities [16].

Schiff bases have been reported for their wide range of biological activities, such as, antitumor [17], anti-tuberculosis [18], antimicrobial [19, 20].

Sulfonamido moiety as privileged chemical entity has been reported to possess antimicrobial [21, 22], antiviral [23], anticancer [24] and antimalarial [25] activities.

The development of new cephalosporins with improved activity against resistant microbes, such as, MRSA, *P. aeruginosa*, is of great interest. The privileged chemical moieties, such as, thiadiazole, Schiff base, cysteine and sulfonamide, have been considered to be incorporated into the C_7 acyl side chain of the cephalosporin molecule to achieve one or more of the desired goals. To the best of the authors' knowledge, sulfonamido moiety has not been incorporated within cephalosporins molecules, so far.

Experimental Section

General methods

Melting points were determined (uncorrected) using electrical melting point apparatus, Electro-thermal 9300, USA. The infrared spectra were performed in KBr disc by FT- IR spectrophotometer/ Shimadzu. Elemental micro-analyses (CHN) were performed by Euro-vector EA 3000A, Italy. ^{1}H-NMR spectra was recorded using NMR Bruker 500 MHz−Avance III and chemical shifts were recorded in parts per million (ppm). ^{1}H-NMR and CHN analyses are kindly performed by faculty of science/University of Jordon. Tetramethylsilane was used as reference.

Chemical synthesis

The synthesis of the target compounds of series 1 and 2 (**1a-d** and **1-3**) were achieved, as illustrated in schemes (1-3).

Chemical synthesis of compounds of series1

5,5'-disulfanediylbis(1,3,4-thiadiazol-2-mine) 1a

This compound was synthesized by oxidation of 2-amino-1,3,4-thiadiazole-5-thiol using hydrogen peroxide [10], as illustrated on scheme 1.

Hydrogen peroxide (7.5mmol, 2.67 ml) was added drop wise to a suspension of 5-amino-1,3,4-thiadiazole-2-thiol (7.5mmol, 1g) in absolute ethanol (10 ml) with continuous stirring for 1hr at room temperature. A yellow precipitate was formed, collected by filtration, washed excessively with distilled water and crystallized from hot ethanol and the product was dried in an oven at 70 ºC. The product was collected as yellow powder, yield 92%, m.p. 221-223^{O}C, The IR spectrum (υ, cm^{-1}); 3265, 3088 (NH_2 stretching), 1637 (C=N stretching), 1610 (C=N stretching), 1552, 1505 (NH_2 bending) and 1138 (C-S stretching).

2-Amino-3-((5-amino-1,3,4-thiadiazol-2-yl)-di sulfanyl) propanoic acid 1b

This compound **1b** is prepared by a sulfhydryl-disulfide exchange reaction performed to produce cysteine linked to 5-amino-1,3,4-thiadiazole-2-thiol [10, 26]. This reaction involves a nucleophilic attack (S_N2) of the thiolate anion of cysteine to the disulfide (**1a**) as the electrophile, as shown in scheme 1.

An aqueous solution (20 ml) of cysteine (3.7 mmol, 0.45g) was added to a suspension of **1a** (3.7 mmol, 1g) in potassium chloride solution (2 M, 10ml) and the mixture was

adjusted to pH 7.5 by potassium hydroxide (5%). The mixture was vigorously stirred for 24 hrs at room temperature. The reaction mixture was filtered to remove the unreacted compound **1a** and the filtrate was neutralized with acetic acid (5%) and placed in a refrigerator. The precipitated product was formed, collected and washed with distilled water, recrystallized from ethanol and dried in an oven at 70 °C. The product was collected as white powder, yield 64%, m.p. 252 ^{O}C (decomposed).The IR spectra (υ, cm^{-1}) showed the characteristic bands; 3250-2950 (broad band of OH and NH_2 stretching), 2918 (asymmetric C-H stretching of methylene), 2858 (symmetric C-H stretching of methylene), 1654 (C=O stretching of carboxyl), 1622 (C=N thiadiazole), 1585 (NH_2 bending). The ^{1}H-NMR spectra (δ, ppm); 2.93 (dd, 1H, -CH_2-), 3.12 (dd, 1H, -CH_2-), 3.5 (t, 1H, CH). The elemental analysis for $C_5H_8N_4O_2S_3$; calculated: found; C, 23.8:23.11, H, 3.2:3.36; N, 22.2:22.95.

2-(benzylideneamino)-3-((5-(benzylideneamino) -1,3,4-thiadiazol-2-yl) disulfanyl)propanoic acid 1c

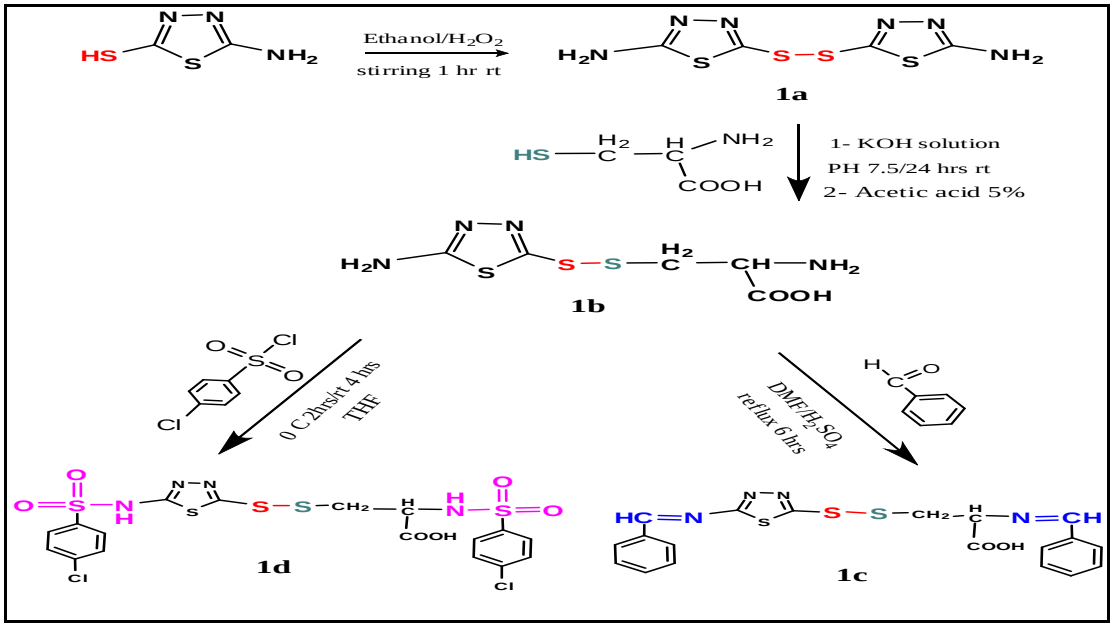
Formation of Schiff bases of 1,3,4-thiadiazole and cysteine **1b** was performed using benzaldehyde in the presence of a catalytic amount (3-4 drops) of concentrated H_2SO_4[27], as illustrated on scheme (1).

Compound **1b** (3.9mmol, 1g) was dissolved in DMF (30ml) containing 3-4 drops of concentrated H_2SO_4 and refluxed with benzaldehyde (7.9mmol, 0.8ml) for 6 h. The solvent was evaporated under vacuum and the residue was washed thoroughly with distilled water, dried in an oven at 70 °C and triturated with petroleum ether (2x10ml) and crystallized from ethanol. This afforded compound **1c**, which was dried in an oven at 70°C. The product was collected as pale brown powder, yield 65%, m.p. 195-197^{o}C. IR spectra (υ, cm^{-1}); displayed the following; 3400-3100 (OH stretching of carboxyl), 1700-1600 (broad band of C=O carboxyl, imines of thiadiazole and imines of Schiff bases. ^{1}H-NMR spectra (δ, ppm); 2.8 (dd, 1H, methylene), 3.09 (dd, 1H, methylene), 3.5 (t, 1H, CH), 7.3-7.78 (t, 1H, C-H and t, 1H, C-H overlapped with d, 1H, C-H and d, 1H, C-H and s, 1H, HC=N). The elemental analysis was calculated for $C_{19}H_{16}N_4O_2S_3$; calculated: found; C, 53.25:54.55, H, 3.76:3.68, N, 13.07:13.31

2-(4-chlorophenylsulfonamido)-3-((5-(4-chloro phenylsulfonamido)-1,3,4-thiadiazol-2-yl)-di sulfanyl) propanoic acid 1d

This is a sulfonamido-thiadiazole compound **1d,** which is synthesized by reacting **1b** with 4-chloro-benzenesulfonyl chloride. This is a nucleophilic attack of the amine groups on the sulfonyl moiety with the liberation of HCl which is neutralized by Na_2CO_3 in the media, as illustrated in scheme (1).

An aqueous solution (30ml) of compound **1b** (3.9mmol, 1g) containing Na_2CO_3 (7.8mmol, 0.82g) was cooled to 0ºC using an ice-bath. 4-Chloro-benzenesulfonyl chloride, (7.8mmol, 1.65g) dissolved in dry tetrahydrofuran (10ml) was added drop wise over a period of 30min and the reaction mixture was continuously stirred for 2hrs at ^{0}C[28]. The mixture was further stirred for 4 hrs at room temperature and the volume was reduced to 10ml and then HCl solution (5%) was added to acidify the mixture to pH 5. The mixture was stored in a refrigerator overnight. The product **1d** was collected as a white precipitate, washed with acetone (2x15ml), recrystallized from ethanol and dried in an oven at 70°C. The product was collected as white powder, yield 77%, m.p. 257-259°C. The IR spectra (υ, cm^{-1}); recorded the following bands; 3200-2900 (O-H stretching of carboxyl, N-H stretching of sulfonamide, C-H stretching of aromatic ring), broad band at 1680-1610 (represents C=O stretching of carboxyl, C=N stretching of imine and C=C aromatic), 1510 (N-H bending of sulfonamide), 1276, 1161 (S=O stretching of sulfonamide). ^{1}H-NMR spectra (δ, ppm), 3.2 (d, 1H, methylene), 3.3 (d, 1H, methylene), 4.2 (t, 1H, CH), 7.57 (s, 2H, chlorophenyl, adjacent to Cl at C2 and s, 2H, chlorophenyl, adjacent to Cl at thiadiazole), 7.79 (s, 2H, chlorophenyl, adjacent to SO2 at C2 and s, 2H, chlorophenyl, adjacent to SO2 at thiadiazole). The elemental analysis was calculated for $C_{17}H_{14}Cl_2N_4O_6S_5$; calculated: found; C, 33.94:35.19, H, 2.35:2.27, N, 9.31:9.92.



Scheme (1) chemical synthesis of compounds series 1

Chemical synthesis of compounds of series 2

7-(2-(benzylideneamino)-3-((5-(benzylidene amino)-1,3,4-thiadiazol-2-yl)disulfanyl)propan amido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid 1

The synthesis of this compound was achieved by reaction of compound **1c** with 7-aminodesacetoxy cephalosporanic acid (7-ADACD) by the mixed anhydride method with ethylchloroformate (ECF) [29] and as shown on scheme 2.

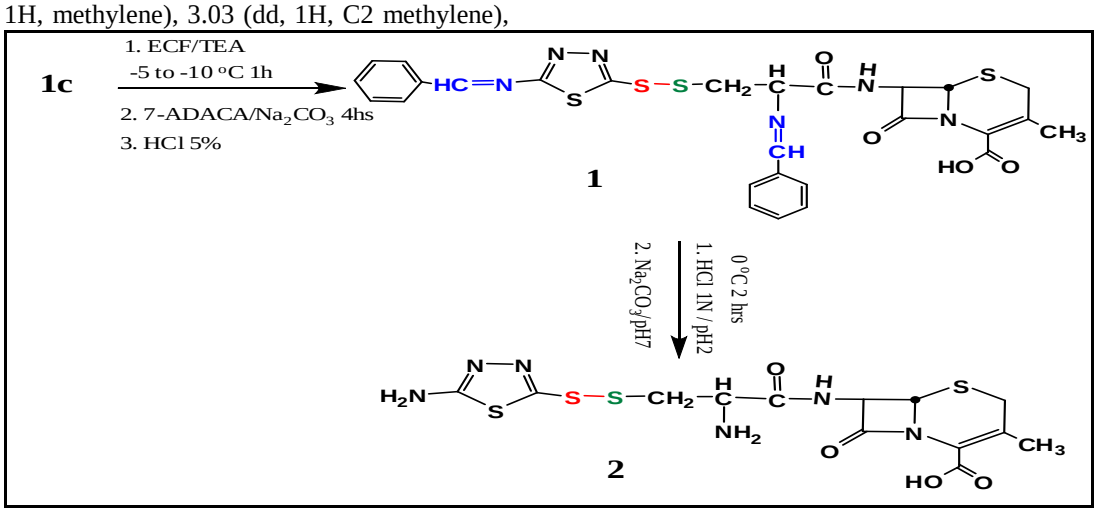
Compound **1c** (2.33mmol, 1g) was dissolved in a mixture (30 ml) of dry acetone and DMF (1:2) containing TEA (2.33mmol, 0.32ml) and the mixture was placed in an ice bath at (-5 to -10°C). A solution of ECF (2.33 mmol, 0.22 ml) was added to the above mixture over a period of 10 min with continuous stirring, which was continued for further 30 min. 7-ADACA (2.33mmol, 0.5g) was dissolved in 10ml of Na_2CO_3 (2.33mmol, 0.24g) solution in distilled water previously cooled to 0°C and was added at once to the above mixture with stirring for 4 hrs. The solvent was then evaporated and the resultant precipitate was washed with HCl solution (3%) and recrystallized from ethanol. The product was collected as brown powder, yield 58%, m.p. 261-263°C. The IR spectra (υ, cm^{-1}); showed the following bands: 3271 (N-H stretching of amide), 3250-2950 (O-H stretching of carboxyl), 3047 (C-H stretching of aromatic ring), 1759 (C=O stretching of β-lactam), 1691 (C=O stretching of carboxyl), 1620-1500 (broad band of C=N of Schiff base, C=N of thiadiazole, C=C of cephem, C=C aromatic ring and N-H bending). ^{1}H-NMR spectra (δ, ppm); 1.83 (s, 3H, CH3), 3.04 (dd,1H, methylene), 3.07 (dd, 1H, C2 methylene), 3.16 (dd,1H, C2, methylene), 3.28 (dd, 1H, methylene), 3.87 (t, 1H, α-CH), 4.96 (s, 1H, C6-H), 5.3 (s, 1H, C7-H), 7.3-7.5 (t, 1H, C-H and t, 1H, C-H overlapped with d, 1H, C-H and d, 1H, C-H of aromatic) 7.79 (s, 1H, HC=N). The elemental analysis was calculated for $C_{27}H_{24}N_6O_4S_4$; calculated: found, C; 51.9:53.76, H; 3.87:3.59, N; 13.45:13.02.

7-(2-amino-3-((5-amino-1,3,4-thiadiazol-2-yl) disulfanyl)propanamido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid 2

This compound was prepared by reaction of compound **1**with HCl solution adjusted at pH 2 [30] and as shown on scheme 2.

Compound **1** (1.6mmol, 1g) was suspended in distilled water (20 ml) and cold HCl solution (1N) was added drop wise and the pH was adjusted to 2 and the mixture was placed in an ice-bath and stirred for 2 hrs. The reaction mixture was neutralized by $NaHCO_3$ solution (5%). The resultant precipitate was excessively washed with distilled water and dried in an oven at 70°C. The product was triturated with petroleum ether (2x10ml) to afford compound **2**. The product was obtained as pale brown powder, yield 50%, m.p. 274-276 °C The IR spectra (υ, cm^{-1}); showed the following bands; 3475-2974 (broad band of O - H carboxyl, NH2 (aliphatic and aromatic)

and N-H amide), 1739 (C=O stretching of β-lactam), 1718 (C=O stretching of carboxyl), 1695 (C=O stretching of amide), 1666 (C=N of imine thiadiazole), 1580-1592, 1510 (broad band of N-H bending of N-H, NH_2). ^{1}H-NMR spectra (δ, ppm), 1.85 (s, 3H, CH_3), 2.86 (dd, 1H, methylene), 3.03 (dd, 1H, C2 methylene), 3.13 (dd, 1H, C2 methylene), 3.16 (dd, 1H, methylene), 3.5 (t, 1H, α-CH), 4.96 (s, 1H, C6-H), 5.3 (s, 1H, C7-H). The elemental analysis was calculated for $C_{13}H_{16}N_6O_4S_4$; calculated: found, C; 34.8:35.93, H; 3.6:3.38, N; 18.74:19.57.

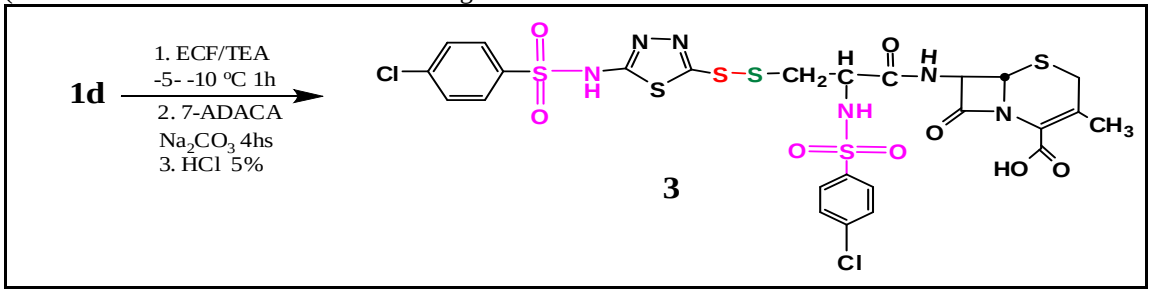


Scheme (2) Chemical synthesis of compounds 1 and 2

7-(2-(4-chlorophenylsulfonamido)-3-((5-(4-chlorophenylsulfonamido)-1,3,4-thiadiazol-2-yl)disulfanyl)propanamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 3

The synthesis of this compound was achieved by reaction of compound **1d** by the mixed anhydride method with ECF [29] and as previously described. The chemical synthesis is illustrated on scheme 3

Compound **1d** (1.25mmol, 1g) was suspended in a mixture (30 ml) of dry acetone and DMF (1:2) containing TEA (1.25mmol, 0.17ml) was placed in an ice bath at (-5 to -10C°). A solution of ECF (1.25mmol, 0.12ml) was added and the mixture was treated as previously described for compound **1**. The product was obtained as white powder, yield 56%, m.p. 291-293°C. The IR spectra (υ, cm^{-1}); 3483 (N-H stretching of amide), 3300-3100 (broad band of O-H and N-H stretching of carboxyl and sulfonamide respectively), 1788 (C=O stretching of β-lactam), 1735 (C=O stretching of carboxyl), 1681 (C=O of amide), 1597 (C=N of aromatic), 1246, 1155 (S=O stretching of sulfonamide). ^{1}H-NMR spectra (δ, ppm); 1.85 (s, 3H, CH3), 2.89(d, 1H, methylene), 3.16 (d, 1H, C2 methylene), 3.19 (d, 1H, C2 methylene), 3.51 (d, 1H, methylene), 3.55 (t, 1H, a-C-H), 4.99 (s, 1H, C6-H), 5.33 (s, 1H, C7-H), 7.49 (s, 2H, chlorophenyl, adjacent to Cl at C2 and s, 2H, chlorophenyl, adjacent to Cl at thiadiazole), 7.73 (s, 2H, chlorophenyl, adjacent to SO_2 at C2 and s, 2H, chlorophenyl, adjacent to SO_2 at thiadiazole). The elemental analysis was calculated for $C_{25}H_{22}Cl_2N_6O_8S_6$; calculated: found; C, 37.64:36.48, H, 2.78:2.93, N, 10.53:10.99.



Scheme (3) Chemical synthesis of compound 3

Antimicrobial evaluation (Determination of MIC values)

The determination of MIC values of the synthesized compounds of series 1 and 2 was achieved using the broth microdilution method [(31)]. A panel of certain microorganisms, such as, *Staph. aureus*, *E. coli*, *P. aeruginosa* and MRSA were used and their stock suspensions measured as 1×10^6 CFU/ml was inoculated. Cephalexin sodium was used as reference standard. The full experimental details are described below:

Broth microdilution method

MIC values of compounds (**1b-d** and **1-3**) were determined against the tested microorganisms using the microdilution method in 96-well plates. An aliquot (150 µl) of the Mueller Hinton broth was used to fill the first experimental well, while the other wells were filled with (100 µl). A aliquot (50 µl) of the tested compound as sodium salt (2mg/ml) was added to the first well to make a total volume of 200µl. Double-fold serial dilution was then carried out across the wells of the plate. The overnight batch culture of the microorganisms (10 µl) was used to inoculate each well to achieve an inoculum size of approximately 1×10^6 CFU/ml. The plates were incubated for 24 h at 37°C. The MIC values were calculated visually according to the degree of turbidity against Mcfarland standard [(31)]. Negative controls (well without sample or reference standard) and positive controls (with cephalexin) were used. Each MIC value was determined in duplicate and the average was calculated and the results are listed on table (1).

Results and Discussion

The FT-IR characteristic bands shown in the spectra of the synthesized compounds were used to identify and confirm their chemical structures depending on the appearance and disappearance of certain chemical groups and consequently their bands. The IR spectrum of compound **1a** (5,5'-disulfanediylbis(1,3,4-thiadiazol-2-amine)), showed sharp peak of the imine at 1637cm^{-1} and very distinguished two absorption bands for the primary amines at 3265cm^{-1} and 3088cm^{-1}. The physical and chemical properties of this compound **1a** confirmed its chemical structure, as it is only soluble in DMF and aqueous acidic solutions and it is insoluble in water, alcohols, chloroform or acetone. The IR spectrum of compound **1b** displayed the characteristic bands of the carboxyl group of cysteine appeared in the range of 3250-2950 cm^{-1}. This broad band overlapped the two amino groups of thiadiazole and the cysteine**.** The appearance of bands at 2918 and 2858cm^{-1} for the asymmetric and symmetric vibration of the methylene and the carbonyl of carboxyl at 1654cm^{-1} of cysteine were clearly recorded. **1b** is insoluble in water and hot ethanol, soluble in DMF and alkaline or acidic aqueous solutions.

The IR spectrum of compound **1c** (Schiff bases of **1b**) displayed a broad band at 1700-1600cm^{-1} which refers to the four imines (two imines of thiadiazole and the two Schiff bases) and the C=O of carboxyl group. Compound **1c** is soluble in ethanol and acetone on contrary of compound **1b**.

The IR spectra of compound **1d** showed broad band at 3200-2900cm^{-1} representing the carboxyl group and the two NH of the sulfonamide group. Appearance of a broad band at 1680-1610cm^{-1} represents the C=O stretching of carboxyl, the C=N stretching of imine and C=C of aromatic. The spectrum also displayed clear bands at 1510cm^{-1} and 1467cm^{-1} for NH bending. The sulfon groups (O=S=O) recorded characteristic bands at 1276 and 1161cm^{-1}, which were not displayed by compound **1b**. Compound **1d** has noticeable water solubility compared with its precursor (**1b**) and this is may be due to the 4-chlorophenylsulfonyl moiety.

The IR spectrum of compound **1** displayed a characteristic band at 3271cm^{-1} representing the NH stretching of amide. A broad band at 3250-2950cm^{-1} may refer to the presence of carboxylic group and characteristic bands at 1759cm^{-1} and 1691cm^{-1} indicate the C=O of β-lactam and C=O of carboxyl respectively. A broad band at 1620-1500cm^{-1} represents C=N of Schiff bases, C=N of thiadiazole, C=C of cephem and NH bending. The physicochemical properties of compound **1** were clearly differentiated from its precursors (7-ADACA and **1c**).

The IR spectra of compound **2,** showed two amino groups (aliphatic and aromatic) and a broad band at 3475-2974 cm^{-1} for the OH of carboxyl, amines and NH of amide. C=O stretching of β-lactam, carboxyl and amide appeared at 1739, 1718 and 1695 cm^{-1} respectively. The liberated free amine groups of compound **2** have enabled the compound to be converted to the hydrochloride salt and separated from the aqueous solution.

The IR spectra of compound **3** displayed the characteristic band at 3483cm^{-1} of NH stretching of amide, a broad band at 3400-3100cm^{-1} represents the OH of carboxyl and NH of sulfonamide. Characteristic bands of the β-lactam, C=O of carboxyl and C=O of amide are displayed at 1788, 1735 and 1681cm^{-1} respectively. C=N of thiadiazole appeared at 1597cm^{-1} and the sulfon groups (O=S=O) recorded characteristic bands at 1246 and 1155cm^{-1}.

^{1}H-NMR spectra (δ, ppm) of compounds of series 1 and 2 showed the characteristic peaks for the protons, as expected. All the synthesized compounds were prepared as sodium salt and dissolved in D_2O, therefore, protons on O- and N- could not be detected. The ^{1}H-NMR spectra of compounds **1b-d**, **1** and **2** displayed the phenomenon of complex spin-spin splitting [32] with regard to the methylene protons in the cysteine moiety. This phenomenon has led to the appearance of doublet of doublet, which was clearly shown on spectra of these compounds. In the ^{1}H-NMR spectra the chemical shifts of different protons have been distinct and the spin-spin splitting patterns have been straightforward. However, different kinds of protons in a molecule have overlapping signals, as previously reported [33]. Therefore, the five aromatic ring protons gave a complex (overlapping pattern), even though they are not all equivalent. This observation was noticed in compounds **1c** and **1** but was not detected in compounds **1d** and **3**, since these compounds contain 4-chlorophenyl moiety and the positions of the protons are clearly affected by the inductive effect of chlorine atom. ^{1}H-NMR spectra of compounds **1-3** displayed characteristic peaks of the protons of the cephem nucleus and the characteristic peaks appeared for cysteine moiety, as previously shown for compounds **1b-d**. Compounds **1** and **3** displayed the characteristic peaks for the aromatic substitution, while, compound **2** did not display the aromatic protons, simply because the aromatic substitution was removed.

Antimicrobial evaluation (Determination of MIC values)

The MIC values of the synthesized compounds (series 1 and 2) were determined in comparison with cephalexin. Compound **1b** showed no activity against the microorganisms used in a concentration of < 500µg/ml (table 1). Compound **1c** was less potent than cephalexin against *Staph. aureus*, while it has no activity against the other bacteria used. Compound **1d** showed activity only against *E. coli* and was less potent than cephalexin. Notably, 1,3,4-Thiadiazole derivatives showed significant antibacterial activity against *Staph. aureus* and *E. coli* [34, 35].

The new cephalosporins **1** and **3** showed very interesting results (MIC values of 500 and 250 µg/ml, respectively) against *P. aeruginosa* in comparison with cephalexin, which showed no activity at <500 µg/ml. Compounds **1-3** have much higher activity against *E. coli* (15.6-250µg/ml), when compared with cephalexin (250µg/ml). Compound **3** was the most potent (15.6µg/ml) among this series of new cephalosporins against *E. coli*. The new cephalosporins **1-3** showed very interesting activity against *Staph. aureus* and the MIC values ranged between (31.2-62.5µg/ml) in comparison with cephalexin (125µg/ml). Moreover, compound **1** was the most potent of the series with MIC value of 31.2µg/ml. Cephalosporins **1** and **3** showed very promising and satisfactory results (250 and 500 µg/ml, respectively) against MRSA in comparison with cephalexin, which has no activity at all [31] at concentration of < 500µg/ml. Moreover, compound **1** was more potent than compound **3** with a margin of one fold. The detailed antimicrobial results are stated on (table 1).

The interesting activities of the new cephalosporins **1** and **3** against *P. aeruginosa* and MRSA may indicate their resistance against the β-lactamases produced by these microorganisms. This is an expected observation, due to the presence of 2-benzylideneamino or 2-(4-chlorophenylsulfonamido) moieties in the acyl side chain at the α-carbon adjacent to the β-lactam ring. These two moieties may provide steric effect and consequently may be considered as isosteric replacement for the alkoxyimino moiety, which is essential for the protection against β-lactamases.

It is worth mentioning that compound **3**, which is a new cephalosporin containing 1,3,4-thiadiazole linked to a sulfonamido moiety comprising a new approach of incorporation of sulfonamide within the cephalosporin molecule.

Table(1): MIC values (µg/ml) of the synthesized compounds

Compound	Pseudomonas aeruginosa ATCC 9027	Escherichia coli ATCC 8739	Staphylococcus aureus ATCC 29213	MRSA ATCC 43300
Cephalexin	-------	250	125	-------
1b	-------	-------	-------	-------
1c	-------	-------	500	-------
1d	-------	500	-------	-------
1	500	62.5	31.2	250
2	-------	250	62.5	-------
3	250	15.6	62.5	500

Keynote: ---- = No growth

Acknowledgement

The authors are very grateful for the University of Baghdad for supporting this research work. The great efforts of Dr. Amal G. Al-Bakri / pharmaceutics and microbiology department / Faculty of pharmacy / University of Jordan, for performing the antimicrobial evaluation are deeply acknowledged.

References

1. Abdulrasool M. M., Jawad A. H. and Shneine J. K., Synthesis, characterization and evaluation of biological activity of new heterocyclic compounds containing 1,2,4- triazole and 1,3,4-thiadiazole Rings. International Journal of Applied Science and Technology, 2012, 2:10, p155-164.

2. Levy Stuart B. and Marshall B., Antibacterial resistance worldwide: causes, challenges and responses, Nature medicine, 2004, 10:12, P. S122-S129.

3. Wilson and Grisvold's textbook of organic medicinal and pharmaceutical chemistry, 12th ed., Lippincott Williams and Wilkins, 2011.page 259.

4. Roger G. "Antibiotic and Chemotherapy: anti-infective agents and their use in therapy", Elsevier health sciences, 8th Ed, 2003; chapter 16, 86-99.

5. Gaurava K., Kundua S. and Srivastavab R., Synthesis and in vitro antibacterial activity of some novel cephem antibiotic. Int. J. Pharm. Pharma Sci., 2012, 4(3), 0975-1491.

6. Burger's, Medicinal chemistry and drug discovery, 6th edition. John Wiley & Sons, Inc. USA, 2003, 610-614.

7. Kasai M., Hatano S., Kitagawa M., Yoshimi A., Nishimura K.,\ Mori N., Sakai A. and Sugihara T., a Novel orally active bifunctional prodrug of ceftizoxime synthesis and relationship between physicochemical properties and oral absorption , Chem . Pharm . Bull. , 1999, 47(8) 1081—1088.

8. Scott.J. Hecker et al; Prodrugs of cephalosporin RWJ-333441 (MC-04,546) with improved aqueous solubility, Antimicrob. Agents Chemother., 2003; 47(6): 2043–2046.

9. Alcaide B. and Aragoncillo C. and Almendros P. "Cephalosporins", Elsevier Ltd, 2008, p. 112-164.

10. Alwan S. M., Synthesis and preliminary antimicrobial activities of new arylideneamino-1,3,4-thiadiazole-(thio/ dithio)acetamido cephalosporanic acids, Molecules, 2012, 17, 1025-1038.

11. Hara K., Matsumoto F., New antimicrobial agent series XXVIII: cefuzonam, US National Library of Medicine National Institutes of Health, 1987; 40(12):1953-63.

12. Demirbas A., Sahin D., Demirbas N. and Karaoglu S. A., Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and investigation of their antimicrobial activities, Eur. J. Med. Chem., 2009,44, 2896–2903.

13. Ishikawa, T., Iizawa, Y., Okonogi, K., Miyake, A. J. Antibiot., 2000, 53, 1053.

14. Chahine E. B. and Nornoo A. O., Ceftobiprole: The first broad-spectrum anti-methicillin-resistant Staphylococcus aureus β-Lactam, J. Exp. Clin. Med., 2011, 3(1):9-16.

15. Carris N., Ceftaroline: A new cephalosporin with activity against methicillin-resistant Staphylococcus aureus, Pharma note,2011, 20:10.

16. Kishi, Y.; Fukuyama, T.; Nakatsuka, S. New method for the synthesis of epidithiodiketopiperazines. J. Am. Chem. Soc., 1973, 95, 6490–6492.

17. F.D.Popp, J. Org. Chem., 1961, 26, 1566.
18. N. Solak, S. Rollas, Arkivoc, 2006, xii, 173.
19. K.N. Venugopala, V.A.Jayashree, Indian J. Pharm. Sci., 2008, 70, 88.
20. S.J. Wadher, M.P. Puranik, N.A.Karande, P.G.Yeole, Int. J. Pharm. Tech. Res., 2009, 1, 22.
21. Genç Y, Özkanca R, Bekdemir Y., Antimicrobial activity of some sulfonamide derivatives on clinical isolates of Staphylococcus aureus, Ann. Clin. Microbiol. Antimicrob., 2008, 7(17):6 pp. DOI:10.1186/1476-0711-7-17.
22. Eshghi H, Rahimizadeh M, Zokaei M, Eshghi S, Eshghi S and Faghihi Z., Synthesis and antimicrobial activity of some new macrocyclic bis-sulfonamide and disulphides, Eur. J. Chem., 2011;2(1):47-50.
23. Ao Z, Jayappa KD, Labine M, Zheng Y, Matthews C, Kobinger G, et al., Characterization of anti-HIV activity mediated by HIV-1 integrase c-terminal domain polypeptides expressed in susceptible cells, J. Antivir. Antiretrovir., 2010;2(1):20-28.
24. Reddy NS, Mallireddigari MR, Cosenza S, Gumireddy K, Bell SC, Reddy EP, et al., Synthesis of new coumarin 3-(N-aryl) sulfonamides and their anticancer activity, Bioorg. Med. Chem. Lett., 2004, 14(15):4093-4097.
25. Miller LH, Baruch DI, Marsh K, Doumbo OK., The pathogenic basis of malaria, Nature.2002; 415(6872):673-79.
26. Bach R.D.; Dmitrenko O. and Thorpe C.: Mechanism of thiolate-disulfide interchange reactions in biochemistry, J. Org. Chem., 2008; 73: 12.
27. Mobinkhaledi A., Jabarpour M. and Hamata A., Synthesis of some novel and biologically active Schiff bases bearing a 1,3,4-thiadiazole moiety under acidic and PTC conditions., J. Chil. Chem. Soc., 2011, 56,3, 812-814.
28. Ajani OO; Familoni OB.; Echeme JO.; Wu F. and Sujiang Z., Synthesis and Antibacterial Activity of N,N-Diethylamide Bearing Benzenesulfonamide Derivatives, Am. Chem. Sci. J., 2013, 3(1): 34-49.
29. Rho, H. S., et al; A Convenient Method for the Preparation of Alkanolamides, Bull. Korean Chem. Soc. 2006, Vol. 27, No. 4, 584-586.
30. Cockerill, F.R., Wikler, M.A., Alder, J. and et al, Performance standards for antimicrobial susceptibility testing, 22th ed., 2012, Clinical and laboratory standards institute, USA, 53.
31. Prasad S. Raje, Preparation of Amino-Protected Lysine Derivatives, United States Patent Application Publication, 2002; Pub. No US2002/0042536A1.
32. John MuMurry, Organic chemistry, 7th ed., Thomas Brooks/cole , 2011, USA, 440-481.
33. Silverstein R. M., Webster F. X., Kiemle D. J., Spectrometric identification of organic compounds, 7th ed., John Wiley & Sons, 2005 ,UAS,144-216.
34. Al-Shihry, S. S., Synthesis and spectral studies of Schiff bases of 2-amino-5-mercapto-1, 3, 4-thiadiazole, Sci. J. King Faisal University, 2005; 6 (2).
35. Hussain S., Sharma S., Amir M., Synthesis and Antimicrobial Activities of 1, 2, 4-Triazole and 1,3, 4-Thiadiazole Derivatives of 5-Amino-2-Hydroxybenzoic Acid, E-J. Chem., 2008; 5(4), 963-968.