Synthesis of Novel AminothiadiazolylCephalosporins

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Abstract

Amino-thiadiazolyl cephalosporins were designed, synthesized and their structures were characterized by spectral and elemental analysis. These cephalosporins included derivatives 7-amino cephalosporanic acid ,acylated at the C7-amino group.The acyl side chain contained 2 amino 5-mercapto-1,3,4,thiadiazole cross-linked with benzaldehyde or *p*-chlorobenzaldehyde through a Schiffs base. The Cephalosprin nucleus was linked with these Schiffs bases through a sulfide linkage using chloroacetic acid as a spacer arm, or through a disulfide bond using mercaptoacetic acid as a spacer arm. These aminothiadiazoyl-Cephalosporins were characterized using spectral and elemental analysis.

A preliminary antimicrobial assay for these novel aminothiadiazoyl-cephalosporins was achieved using representative strains of G(−) and G(+) bacteria and Candida Albicans. The prepared cephalosporins showed reasonable antimicrobial potency when compared with Cephalexin.

Introduction

The most useful modification of the basic 7 amino group in cephalosporanic (or desacetoxy) acids have resulted from acylation of this group with different acids (1-3).These modifications have led to the production of various cephalosporins of different activities. It has been shown that the incorporation of heterocyclic substituents in cephalosporanic acid produces more potent antimicrobial activities (4 ,5). Successful cephalosporins contain at least one of these substituents ,such as cefotaxime or ceforazidine (6). Certain cephalosporins containing 1,2,4-thiadiazole moiety linked at the acyl side chain through an amide linkage were very potent with improved MICs values (7-9). The 1,3,4-thiadiazole and aminothiazole are associated with various biological activities, probably due to the presence of toxophoric –N=C-S- group (10-12). The importance of the sulfur atom in drugs as thioether or disulfide linkages in the acyl side chain provides great stability for the three-dimensional structure of the molecule (13). Besides, these sulfur atoms have great contributions to the antimicrobial activities (14, 15).

B-Lactam antibiotics may be inactivated by bacterial enzymes (acylases and *B*-lactamases) . Based on this fact, the developments of this bacterial resistance has initiated the search for novel cephalosporins that may have reasonable potency and sensitivity against such microbes.

In view of these observations , novel aminothiadiazolylcephalosporins (compounds V,VI , XI and XII) were designed ,synthesized ,characterized and evaluated for their antimicrobial activities when compared with cephalexin as a standard.

Experimental Section

Materials;

7-Aminocephalosporanic acid was a kind gift from Al-Nejma bulk pharmaceutical co.,Jordan. Benzaldehyde, p-chlorobenzaldehyde and 2-Amino-5-mercapto-1,3,4-thiadiazole were from Fluka. All other chemicals and solvents are analar grade.

Melting points were measured (uncorrected) in capillaries by Thomas Hoover melting point apparatus. IR spectra were recorded by Perkin Elmer 1310 IR spectrophotometer. Elemental microanalyses were done by C.H.N analyzer,type 1106,Carlo Erba.

Chemical Methods :

The target compounds, aminothiadiazolyl cephalosporins, and their precursors are synthesized as illustrated for each case in scheme 1 and 2.

Preparation of 5-[1-arylideneamino]-2-mercapto-1,3,4-thiadiazole (compounds I and II)

These compounds were prepared by reaction of benzaldehyde or *p*-chlorobenzaldehyde with 2-amino-5-mercapto-1,3,4-thiadiazole the general Schiffs base reaction (16) as illustrated in scheme 1.

Procedure;

2-Amino-5 mercapto 1,3,4 thiadiazole (0.01M,1.33g) was refluxed with benzaldehyde (0.01M,1.06g) in absolute ethanol (50ml) for 6hr. The reaction mixture was cooled and the product was precipitated, filtered washed with cold ethanol and recrystallized to give compound I. The reaction was repeated using *p*-chlorobenzaldehyde and treated similarly as above to obtain compound II .The

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percent yield ,M.P. and Rf values are presented on Table (1).

Preparation of 2-[2-(1-arylideneamino)-1,3,4-thiadiazole-5-yl]-thioacetic acid (compounds III and IV)

These compounds were prepared through the S-alkylation reaction using chloroacetic acid (17) as shown in scheme 1.

Procedure:

To a solution of compound I (14.7mM,4.7g) in ethanol (50ml) ,potassium hydroxide (14.7mM) and chloroacetic acid (14.7mM ,1.4g) were added and the mixture was refluxed for 2hr. The hot mixture was filtered and the ethanolic solution was evaporated under reduced pressure . The residue was dissolved in distilled water , acidified with diluted hydrochloric acid to pH 3. A precipitate was collected by filtration ,washed with cold distilled water and dried in an oven to provide compound III .A similar procedure was performed using compound II to prepare compound IV .The percent yield ,M.P. and Rf values are given on Table 1

Preparation of 7-[2-(2-(1-arylideneamino)-1,3,4-thiadiazole-5yl)-thioacetamido]-Cephalosporanic acid (compounds V and VI)

These novel aminothiadiazolylCephalosporins were prepared using the mixed anhydride method (18) and as illustrated in scheme 1.

Procedure:

An anhydrous solution of compound III (11.1mM, 4.7g) and triethylamine (11.4mM ,1.15g) in THF (30ml) was cooled to -10C . To this solution an aliquot of thionyl chloride (11.4mM, 1.35g) was added drop wise with continuous stirring . The resulting mixture was left for 30min with continuous stirring at 0C . A cold solution of 7-aminocephalosporanic acid (11.4mM, 2.6g) and triethylamine (11.4mM, 1.15g) in distilled water (10ml) was added to the above mixture. The mixture was vigorously stirred for 2hr at room temperature, diluted with distilled water (30ml) and then was extracted with diethylether (2x20ml) . The aqueous phase was acidified with diluted HCl to pH 3, and was extracted with ethylacetate (3x20ml) . The extracts were pooled together, dried over anhydrous sodium sulfate and then evaporated to dryness under reduced pressure. The residue was triturated with petroleum ether and collected to provide compound V . A similar procedure was conducted using compound IV to yield compound VI . The percent yield , M.P. and Rf values are listed on Table 1. The elemental microanalysis (C.H.N.) for these new amino thiadiazoylcephalosporins are presented on Table (2).

The IR spectra of the characteristic bands are as follows:

3100 (NH), 1790(C=O lactam), 1740 (C O ester group), 1720 (C O amide), 1635 (C=N Schiffs base), and 1595 (C=C arom.)

Preparation of 5,5-Dithio-[2-(1-arylideneamino)-1,3,4,-thiadiazole. (compounds VII and VIII)

These compounds were prepared by oxidizing compounds I and II using hydrogen peroxide. scheme 2.

Hydrogen peroxide (14.8mM, 3%) was added drop wise to a solution of compound I (22mM, 4.5g) in ethanol (50ml) with continuous stirring for 1hr at room temperature (19). A white precipitate was obtained, collected by filtration, washed excessively with distilled water and dried in an oven at 70C. This represent compound VII .

A similar method was conducted using compound II and treated as above leading to the formation of compound VIII The percent yield , M.P. and their Rf values are given on Table 1.

Preparation of 2-[2-(1-arylideneamino)-1,3,4-thiadiazole-5-yl]-Dithio-acetic acid (compounds IX and X)

A thiol --disulfide exchange reaction was employed in the preparation of these compounds (20), as illustrated in scheme 2.

Procedure:

An aqueous solution (30ml) containing mercaptoacetic acid (7.4mM) and potassium hydroxide (7.4mM, 0.42g) adjusted to pH 7.5 was added to a suspension of compound VII (7.4mM,) in potassium chloride (25ml,2N) at pH 7.5. The mixture was then acidified with dilute HCl and the precipitated product was collected by filtration ,washed thoroughly with distilled water and dried in an oven at 70C. This method has resulted in the formation of compound IX . Compound X was prepared using similar procedure starting from compound VIII.

Their percent yields ,M.P. and Rf values are presented on Table 1.

Preparation of 7-[2-(1-arylideneamino)-1,3,4-thiadiazole-5-yl-(dithioacetamido)]- Cephalosporanic acid (compound XI and XII)

These aminothiadiazoylcephalosporins containing disulfide bonds were prepared using the mixed anhydride method (18) as previously described. Scheme 2 illustrate the chemical pathway. Their percent yield , M.P. and Rf values are listed on Table (1). Elemental microanalysis (C.H.N.) for these compounds XI and XII are presented on Table (2).

The IR spectra of the characteristic bands are as follows.

3100 (N-H), 1785(C O lactam), 1735 (C O carboxylic), 1710 (C=O amide),

1640 (C- N schiff's base) and 1605 (C C arom.).

Preliminary Microbiological Assay

The antimicrobial activities of the newly synthesized amino-thiadiazolyl-cephalosporins were determined by the agar diffusion method (21) using representative G(−) ,G(-) bacteria and Candida Albicans on Tryptic soya agar media. The newly prepared cephalosporins were dissolved in dimethylsulfoxide leading to concentrations of 1ug/ ml. Cephalexin was used as the standard and the activities were presented as inhibition zones for each compound (Table 3).

Determination of the Minimum inhibitory Concentrations (MIC) were also achieved using standard two-fold dilution method (22). A suspension of various microorganisms from sterile overnight cultures on Tryptic soya broth media was prepared by dilution with sterile distilled water (1:100).

The results of the antimicrobial assay (MIC values) are presented on Table (4).

Results and Discussion.

The synthesis of aminothiadiazolyl-cephalosporins derived from 7-aminocephalosporanic acid and 2-amino-5-mercapto-1,3,4-thiadiazole were carried out (scheme 1 and 2). The acyl side chain of these cephalosporins contain a sulfide (scheme 1) and disulfide bonds (scheme2). The incorporation of an amino thiadiazole group together with the disulfide bond contribute greatly to the stability of the molecule (13) and add antimicrobial activity (15). The expected dual action of the cephalosporin nucleus and the aminothiadiazole ring , together with sulfide or the disulfide linkages, is based on the possible synergism resulting from a simultaneous action of these moieties against different penicillin binding proteins(PBP) (23). Heterocyclic amines (24,25) have been widely used for the synthesis of new schiff's bases. Accordingly, compounds I-IV were prepared and used as one of the starting materials for the preparation of the target compounds.

The presence of a Schiffs base in these cephalosporins may also contribute in providing greater stability.

However, these new compounds showed reasonable antimicrobial activities compared with cephalexin, particularly compounds XI and XII .They showed even better activities against staphaur. and E.coli. It is worth trying on resistant microbes to have full spectrum of activities of these new cephalosporins, since they may have certain sensitivities against resistant microbes. They have bulky groups surrounding the B-lactam containing different linkages and groups. The MICs values indicated the need of higher concentrations than what needed for cephalexin and this may account for the large molecules we are getting and this may affect the diffusion of these molecules into the agar and also the cell membrane of microorganisms.

Generally, the more hydrophilic compounds showed the higher outer membrane permeability (26), although small hydropathic molecules can penetrate the cell membrane. The outer membrane of G (-) bacteria is believed to be a barrier to the penetration of B-lactam antibiotics.

The above in vitro preliminary antimicrobial activities are only an indication for certain parameters and evaluation and a proper and detailed study including resistant microbes are necessary to evaluate the actual activities.

Table(1). The percent yield, M.P. and Rf values of the Aminothiadiazolyl cephalosporins and their precursors

Compound	Yield %	M.P (C°)	Rf Values +		
			S_1	S_2	S_3
I	9[illegible]	88 – 89	0.73	0.64	0.20
II	90	180 – 182	0.60	0.55	0.22
III	66	201 – 203*	0.79	0.82	0.40
IV	50	244 – 246	0.75	0.20	0.45
V	53	175 – 178*	0.83	0.86	0.25
VI	48	140 – 142	0.81	0.78	0.38
VII	46	59 – 61	0.65	0.58	-
VIII	40	216 – 218	0.62	0.42	-
IX	85	230 – 232	0.72	0.62	0.40
X	77	250 – 252	0.61	0.68	0.48
XI	50	302 – 304*	0.79	0.83	0.30
XII	50	222 – 224*	0.70	0.76	0.31

* Decomposed

+ Solvent System: S_1= Ethanol : Acetic acid : Water (2 : 1 : 2)
S_2 = Ethanol : Butanol (2 : 2)
S_3= Chloroform : Ethanol (4 : 1)

Table(2) Elemental Microanalyses of the Aminothiadiazolyl cephalosporins.

Compound	Chemical Formula	M.Wt.	Elemental Analysis					
			Found %			Calculated %		
			C	H	N	C	H	N
V	$C_{21}H_{19}N_5S_4O_4$	533	47.28	3.56	13.13	47.21	3.58	13.1
VI	$C_{23}H_{23}N_5S_3O_4$	529	44.64	3.77	13.34	44.62	3.69	13.2
XI	$C_{21}H_{17}N_5S_4O_3$	515	44.90	3.59	12.59	44.95	3.18	13.1
XII	$C_{23}H_{21}N_5S_3O_3$	511	43.91	3.60	11.68	42.67	3.82	13.1

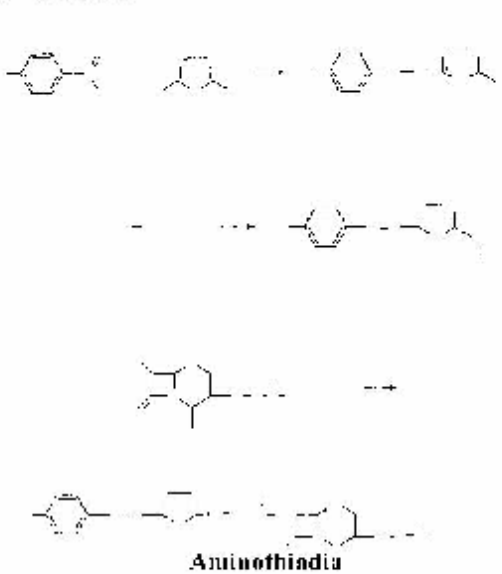
Table (3) Antimicrobial activities of the newly synthesized Aminothiadiazolyl cephalosporins.

Compound	Antimicrobial Activity(zone of inhibition mm)			
	Micrococcus letus(9341)	Staph.aur (25923)	E.Coli (29522)	Pseudo aerug.(27853)
	50 µg / hole			
V	12	16	23	-
VI	8	24	27	3
XI	9	15	25	5
XII	6	25	25	6
Cephalexin	20	18	18	-
	80 µg / hole			
V	17	18	25	-
VI	10	26	28	6
XI	12	18	27	-
XII	9	27	28	9
Cephalexin	26	22	20	-

Table (4) In vitro Antimicrobial activities (MICs values, µg / ml) of the newly synthesized Aminothiadiazolyl cephalosporins.

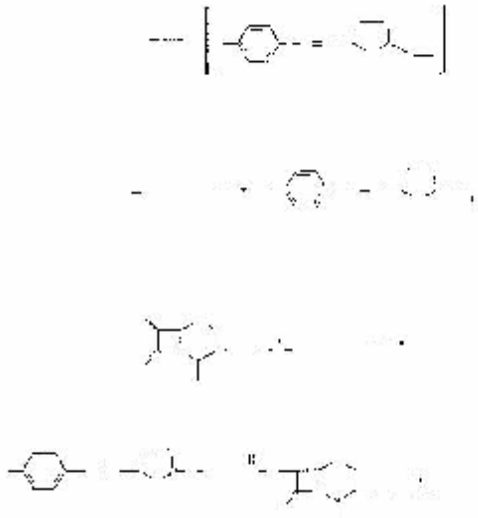
Compound	Micrococcus letus(9341)	Staph.aur. (25923)	E.Coli (29522)	Pseudo. aerug (27853)	Candida albica (10231)
V	25	55	>100	N.T	>100
VI	20	40	50	>100	>100
XI	24	50	80	N.T	>100
XII	6	25	28	N.T	>100
Cephalexin	10	25	30	N.T	N.T

N.T = Not Tested



Aminothiadia

Scheme 1



Aminothiadiazolyl cephalosporins
Scheme 2

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