Synthesis and Antibacterial Properties of 7β-[2-(3-Heteroarylthiazolo[2,3-*c*]-*s*-triazol-5-yl)-acetamido]cephalosporanic Acid Derivatives

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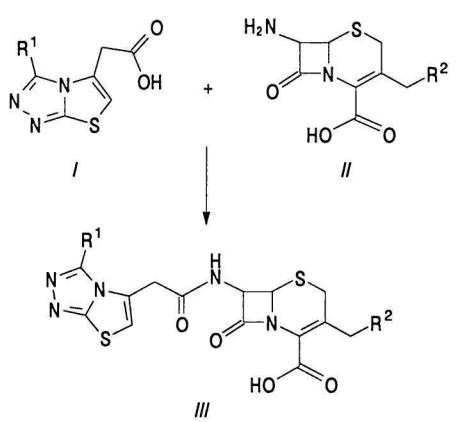
Received 8 January 1993

A series of 7β-[2-(3-heteroarylthiazolo[2,3-*c*]-*s*-triazol-5-yl)acetamido]cephalosporanic acid derivatives were synthesized and evaluated microbially. Although less active than cefotaxime, the compounds having thiazolo[2,3-*c*]-*s*-triazole ring showed good antimicrobial activity against a wide variety of gram-positive and gram-negative bacteria.

Cephalosporin derivatives bearing a thiazole ring are extraordinarily effective antibiotics [1, 2]. In contrast to our knowledge very little has been reported on the related cephalosporins with condensed heterocyclic moieties at the position 7 in the side chain [3—7].

In a previous paper we reported on the synthesis and antibacterial activity of cephalosporins bearing a thiazolo[3,2-*b*]-*s*-triazole moiety at the C-7 position of the cephem ring [8]. They possess a wide spectrum of antimicrobial activity against gram-negative bacteria and increased activity against gram-positive bacteria [9]. In this report we describe the synthesis, physicochemical properties, and antimicrobial activities of cephalosporin derivatives having the 2-(thiazolo[2,3-*c*]-*s*-triazol-5-yl)acetamido side chain.

Preparation of the 7β-[thiazolo[2,3-*c*]-*s*-triazol-5-yl-acetamido]cephem derivatives *III* listed in Table 1 was performed by acylation of silylated 7-aminocephalosporanic acid or its derivatives *II* as illustrated in Scheme 1. The acylation performed by conventional methods, for example the mixed anhydride method using isobutyl chlorocarbonate or the activated ester method using dicyclohexylcarbodiimide failed. Finally the desired compounds (*IIIa—IIIk*) were obtained when corresponding acyl chlorides of heteroaromatic acetic acids *I* were used (method *A*) or when *N*-methylmorpholine and pivaloyl chloride were used in place of triethylamine and isobutyl chlorocarbonate in the mixed anhydride method (method *B*).



I *II* *III*

Scheme 1

The structures of derivatives *III* were confirmed on the basis of IR and NMR spectral data as shown in Table 1. The heteroaromatic acetic acids *I* used in this study were prepared by intramolecular cyclodehydration of 2-(2-aroylhydrazino)thiazoles, together with trisubstituted *s*-triazoles as was previously reported [10].

The minimum inhibitory concentration (MIC) values of this series of cephalosporins against selected strains of gram-positive and gram-negative bacteria were determined by the standard serial two-fold agar dilution method. Cefazolin (CFZ) and cefotaxime (CTX) were used as reference compounds. Antimicrobial *in vitro* activities of newly synthesized cephalosporins are listed in Table 2. These cephalosporins are as active against the gram-positive organisms, *i.e. Staphylococcus aureus*, *Bacillus subtilis*, *Sarcina lutea*, *Bacillus cereus* as CFZ or CTX, but they are less active than the isomeric thiazolo[3,2-*b*]-*s*-triazole cephem derivatives.

The cephems *III* were more active than CFZ but less active than CTX against *S. pyogenes*.

Compounds *III* were less active than CTX against the gram-negative bacteria: *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Enterobacter cloacea*, their activity is similar to that of CFZ. By the displacement of the 3-acetoxy group with heteroaromatic thiols no enhancement of the antibacterial activity against gram-negative bacteria was observed. From these results it can be seen that

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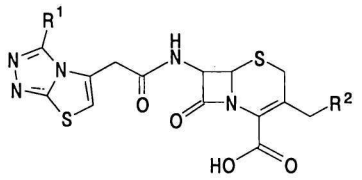
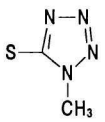
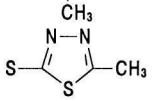
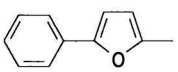
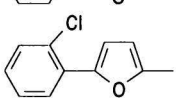
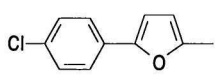
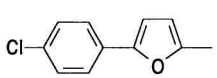
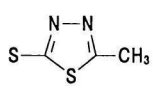
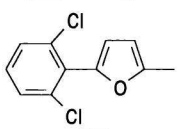
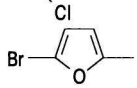
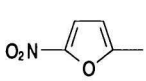
Table 1. Characterization of the Prepared Compounds



Compound	R^1	R^2	Yield %	M.p. °C	IR (KBr) β-lactam $\tilde{\nu}$/cm^{-1}	^{1}H NMR (DMSO-d_6), δ		
						H_{Het} s	C-7—H dd,1H J = 5.8 Hz	C-6—H dd,1H J = 5 Hz
IIIa		$OCOCH_3$	71[a]	172—175	1768	7.36	5.97	4.99
IIIb			75[a]	130—134	1775	7.41	5.92	4.76
IIIc			85[b]	188—192	1768	7.40	5.87	4.92
IIId		$OCOCH_3$	38[b]	190—193	1775	7.42	5.42	5.01
IIIe		$OCOCH_3$	42[b]	212—215	1770	7.37	5.82	4.81
IIIf		$OCOCH_3$	33[b]	209—211	1770	7.37	5.91	4.72
IIIg			49[b]	215—218	1765	7.35	5.76	4.88
IIIh		$OCOCH_3$	50[a]	180—183	1775	7.50	5.78	4.73
IIIi		$OCOCH_3$	68[a]	127—130	1765	7.48	5.90	5.00
IIIj		$OCOCH_3$	67[a]	202—205	1770	7.35	5.92	4.73
IIIk		$OCOCH_3$	21[a]	178—180	1778	7.50	5.86	4.97

Active derivative: *a*) acid chloride, *b*) mixed anhydride. Elemental analyses were in satisfactory agreement with calculated values.

these compounds are distinctly less active than the corresponding thiazolo[3,2-*b*]-*s*-triazole cephalosporin derivatives.

Of our compounds, entry *IIIj* proved to be the most active against gram-negative as well as gram-positive organisms.

EXPERIMENTAL

Melting points were determined using a Boetius micro hot-stage apparatus. IR spectra were measured on a Perkin—Elmer 377 spectrophotometer. ^{1}H NMR spectra were run with Jeol FX-100 instrument using TMS as internal standard. Solvent evaporations were performed under reduced pressure at temperatures below 40 °C.

7β-[2-(3-Heteroarylthiazolo[2,3-*c*]-*s*-triazol-5-yl)]acetamidocephem Derivatives *IIIa—IIIk*

Method A

To a suspension of PCl_5 (2.08 g; 10 mmol) in meth-

Table 2. Antimicrobial Activities of Cephalosporins

Compound	MIC/(μg cm^{-3})									
	B.s.	B.c.	S.l.	S.a.	S.p.	En.c.	Es.c.	P.m.	P.v.	K.p.
IIIa	0.125	0.125	–	0.5	4	–	0.03	–	64	–
IIIb	0.125	0.125	0.125	4	4	–	0.125	> 128	32	16
IIIc	0.125	0.125	0.25	0.125	4	> 128	0.5	> 128	16	16
IIId	0.5	0.5	–	0.125	4	–	0.25	32	32	–
IIIe	0.125	0.5	–	0.25	4	–	0.5	32	–	–
IIIf	0.125	0.06	0.03	0.03	0.5	64	0.125	32	32	32
IIIg	0.125	0.125	0.25	0.125	1	64	0.25	> 128	32	32
IIIh	0.125	0.125	0.125	0.125	2	–	0.5	> 128	32	32
IIIi	0.5	0.5	0.25	0.25	8	–	0.5	–	–	> 128
IIIj	0.125	0.06	0.125	0.125	2	–	0.125	0.5	4	32
IIIk	0.25	0.125	0.03	0.125	4	–	0.25	0.5	32	32
CTX	0.125	0.125	0.125	0.125	0.125	12	0.125	0.125	1	0.5
CFZ	0.125	0.125	0.5	0.5	> 128	–	1	8	64	32

Abbreviations: B.s. — *Bacillus subtilis* CCM 1999, B.c. — *Bacillus cereus* CCM 869, S.l. — *Sarcina lutea* IEM Sar 5/58, S.a. — *Staphylococcus aureus* IEM Aur. 78/71, S.p. — *Streptococcus pyogenes* IEM A1/49, En.c. — *Enterobacter cloacae* CCM 2320, Es.c. — *Escherichia coli* IEM Eck 67/59, P.m. — *Proteus mirabilis* IEM Prmi 7/49, P.v. — *Proteus vulgaris* CCM 1799, K.p. — *Klebsiella pneumoniae* CCM 1848, CTX — cefotaxime, CFZ — cefazolin. Agar dilution method with Mueller—Hinton agar at 37 °C for 18 h, inoculum size 10^6 cfu cm^{-3}.

ylene chloride (20 cm^3) 3-heteroarylthiazolo[2,3-*c*]-*s*-triazol-5-ylacetic acid (10 mmol) was added with ice-cooling, and the reaction mixture was stirred at this temperature for 1 h. To a solution of 7β-amino-3-(acetoxymethyl)- or -3-(heteroarylthiomethyl)ceph-3-em-4-carboxylic acid (10 mmol) and *N*-(trimethylsilyl)acetamide (7.92 g; 60 mmol) in methylene chloride (80 cm^3) the above acylchloride solution was added at –15 °C, and the reaction mixture was stirred at –15—0 °C for 1 h. To the reaction mixture a mixture of ethyl acetate and water was added and the organic layer was separated. After water was added to the organic layer, pH of the mixture was adjusted to 7.5 with $NaHCO_3$ solution. Organic layer was dried ($MgSO_4$). The solvent was concentrated to 1/3 volume and diethyl ether was added. The resulting precipitate was filtered off, washed with diethyl ether and dried (P_2O_5).

Various cephalosporin derivatives *IIIa—IIIk* prepared by method *A* are listed in Table 1.

Method B

To a stirred solution of 3-heteroarylthiazolo[2,3-*c*]-*s*-triazol-5-ylacetic acid (10 mmol) in dry dimethylformamide (5 cm^3), cooled to – 5 °C *N*-methylmorpholine (1.2 cm^3; 11 mmol) followed by a solution of pivaloyl chloride (1.23 cm^3; 10 mmol) in dry dichloromethane (20 cm^3) were added.

After stirring for 30 min at – 5 °C a solution of 7β-amino-3-(acetoxymethyl)- or -3-(heteroarylthiomethyl)ceph-3-em-4-carboxylic acid (10 mmol) and *N*-(trimethylsilyl)acetamide (7.9 g; 60 mmol) in dry methylene chloride (80 cm^3) was added in one portion. After stirring for 1 h at 5 °C and for 2 h at room temperature water was added to the reaction mixture. The mixture was adjusted to pH 2 with 20 mass % H_2SO_4 with stirring. Organic layer was separated, dried ($MgSO_4$) and evaporated to give a solid product, which was then treated with a mixture of ethyl acetate—diethyl ether (φ_r = 1 : 1, 50 cm^3). The solid was filtered off and dried. Characteristic data are presented in Table 1.

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Translated by M. Veverka