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SYNTHESIS OF CEPHALOSPORINS CARRYING ISOXAZOLYL ACETAMIDO AND RELATED SIDE CHAINS

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Starting from 3,5-dimethylisoxazole the carboxylic acids I and V, the amino acids VIII (L-) and IX (D-), and the ureido acids X (L-) and XI (D-) were prepared, which were used for the synthesis of the new cephalosporins XVIIb, XXa—c (L-), and XXIb (D-). The *in vitro* antibacterial activity of these semi-synthetic antibiotics was studied. The resorption of XVIIb was investigated in mice.

During the past decades numerous semi-synthetic cephalosporins carrying an alkyl-, alkenyl-, or a heteroarylthioacetamido sidechain have been synthesized [1-7], and the preparation of oxa-analogues with a related structure has also been reported [4]. Of these semi-synthetic antibiotics only *Cefotetan* is introduced to medicinal therapy (Table 1). It is surprising, however, that relatively less cephalosporin molecules functionalized with an oxazolyl- [8, 9] or isoxazolyl group [10, 11] at positions 3 or 7 have been prepared, despite the fact that the therapeutic value of similar semi-synthetic penicillins (e.g. *Oxacillin*, etc.) has been long recognized.

To fill in this gap, the present paper reports our studies on the chemical synthesis and *in vitro* structure-antibacterial activity relationship of new, diversely substituted isoxazolylacetamido- and thioacetamido cephalosporins.

CHEMISTRY

The preparation of 3,5-dimethylisoxazol-4-ylacetic acid (I), suitable for the acylation of the cephalosporin nucleus, was carried out [12] in three steps (Scheme 1) from 3,5-dimethylisoxazole (II), and the product was identical with that reported by March in 1901 [13]. Besides the ^{1}H-NMR data [200 MHz, $CDCl_3$, δ ppm: 2.25 and 2.38 (3H, s, 3- and 5-CH_3); 3.38 (2H, s, —CH_2—); 8.80 (1H, br, COOH)], the structure of acid I was also supported by transformation into the crystalline 3,5-dimethylisoxazol-4-ylacetic acid N-hydroxysuccinimine ester III in excellent yield, which was then used for the acylation.

5-Bromomethyl-4-chloro-3-methylisoxazole (IV), required for the introduction of various isoxazol-5-ylthioacetamido side chains, was also obtained from II [14]. Treatment of bromide IV with thioglycolic acid in 80% aqueous acetone in the presence of triethylamine (20°C, 24 h) readily furnished 5-(carboxymethylthio)methyl-4-chloro-3-methylisoxazole which was isolated in form of the potassium salt V. Oxidation of the latter with hydrogen peroxide in glacial acetic acid afforded 5-(carboxymethylsulfonyl)methyl-4-chloro-3-methylisoxazole (VI).

The reaction of IV with L-cysteine (VII; $R^2 = R^3 = H$) and D-penicillamine (VII; $R^2 = R^3 = CH_3$) was carried out in abs. ethanol in the presence of sodium methoxide (20 °C; 1 h), and the new amino acids VIII (L-) and IX (D-), respectively, were isolated after acidification (pH ~5) of the reaction mixture. Treatment of acids VIII and IX with potassium cyanide in refluxing water at pH ~2 gave the highly crystalline α-ureido compounds X (L-) and XI (D-), respectively. The reaction of VIII with Boc_2O (20°C; 2 h) [15] yielded the N-Boc-amino acid XII as a brown syrup, which was used without further purification. The characteristic physical data of the compounds described above are collected in Table 2.

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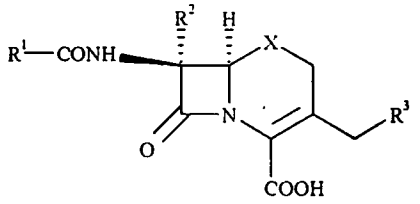
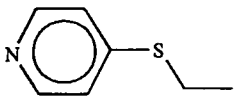
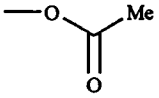
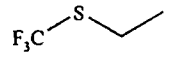
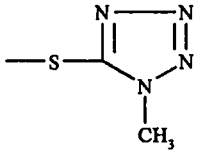
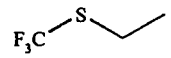
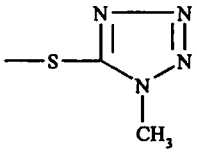
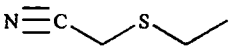
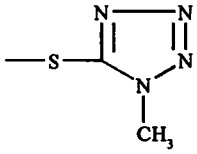
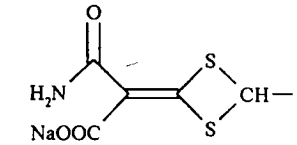
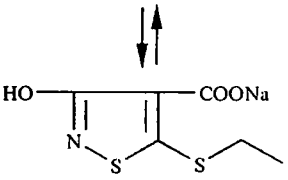
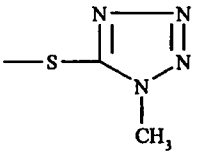
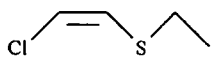
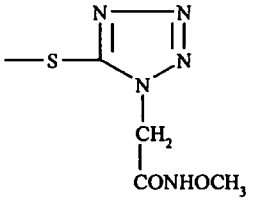
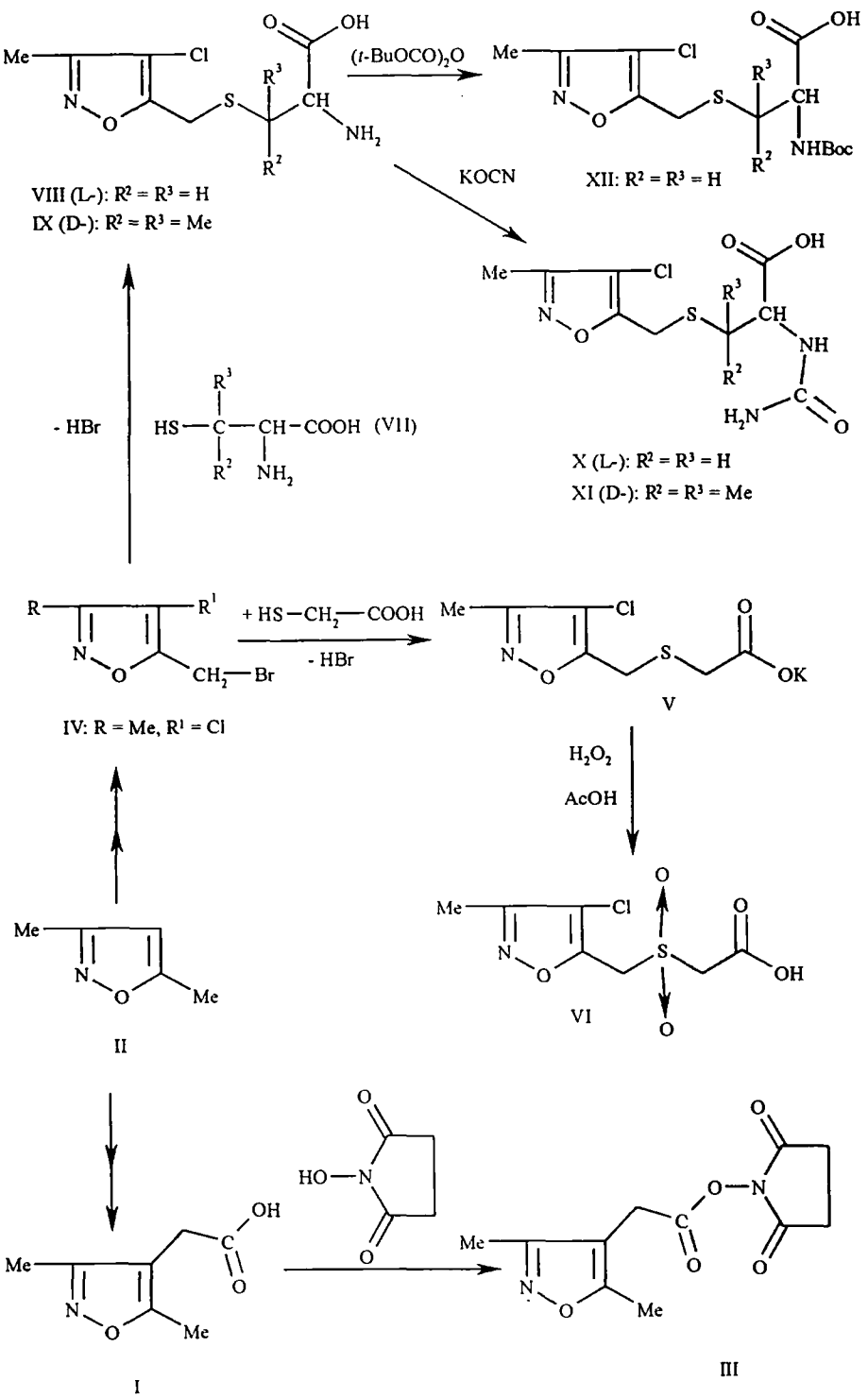
TABLE 1. Cephalosporins Carrying an Alkyl-, Alkenyl-, or a Heteroaryl-Thioacetamido Side Chain



Designation	R^1	X	R^2	R^3	Literature
Cephapirin		S	H		1, 7
Cefazaflur (SKF-59962)		S	H		1, 2, 5
SKF-73678		S	OMe		
Cefmetazol (CS-1170)		S	OMe		1,2
Cefotetan	 	S	OMe		2
Shionogi compound		O	OMe		4

Scheme 1



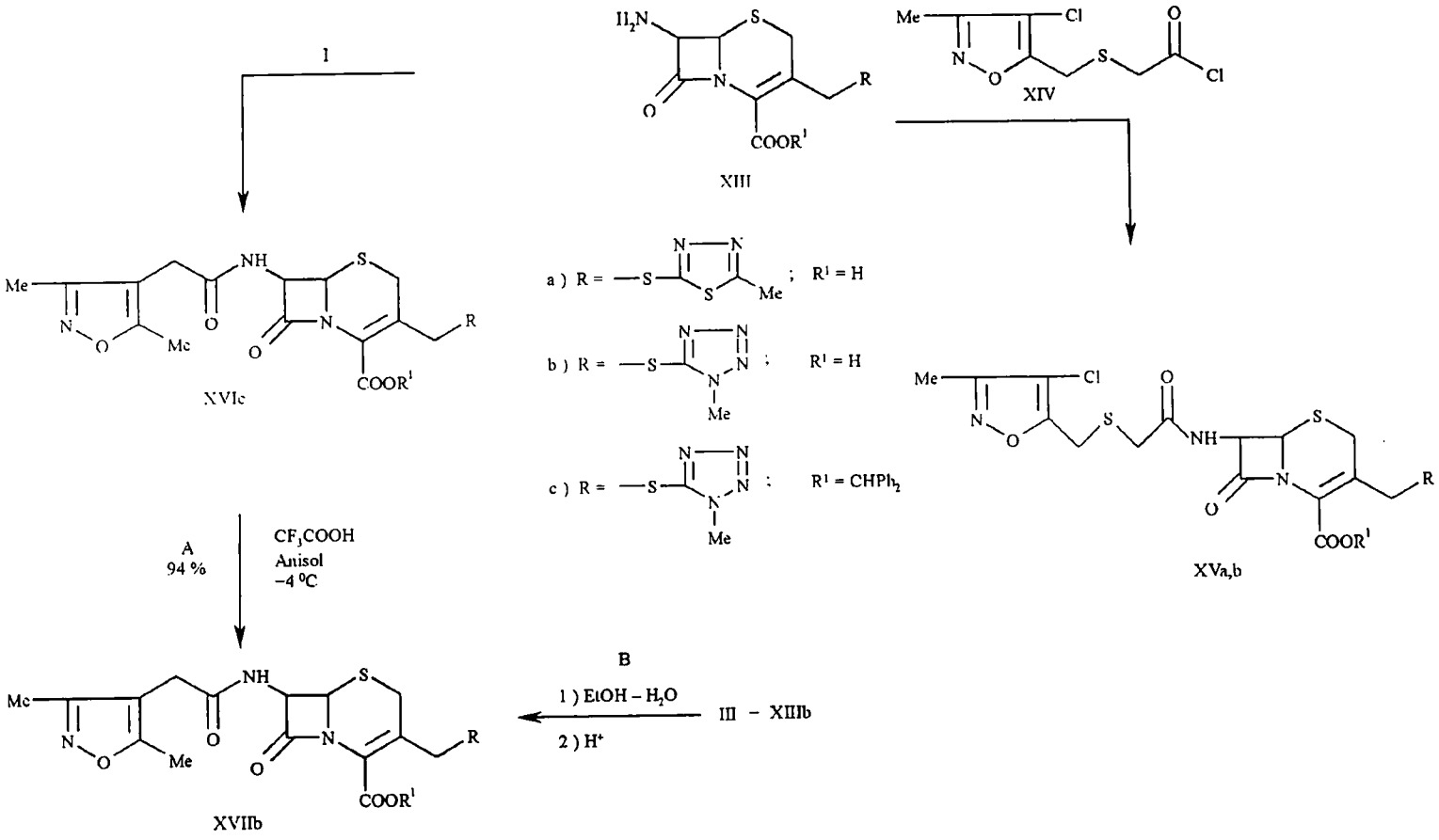
The cephalosporins XIIIa-c, used for the synthesis of the new β-lactam molecules, were prepared by known methods [16]. Among them XIIIa and XIIIb are most particularly interesting due to their diminished metabolic ability. Acylation of XIIIa,b with the acid chloride XIV in 50% aqueous acetone in the presence of sodium hydrogen carbonate (0-5°C) furnished the semi-synthetic cephalosporins XVa,b, respectively, which were isolated in the form of the potassium salts (Scheme 2).

TABLE 2. Physicochemical Properties of the Prepared Izoxazole Compounds

Compound	R^2	R^3	Yield, %	Mp, °C[a]	Molecular formula	Found, % / Calculated, %			$[\alpha]_D^{20}$
						N	S	Cl	
I			42 (overall)	118...119 Lit [14] 122 (B)		—	—	—	
III			93	122...123 (D)	$C_{11}H_{12}N_2O_5$	10.85 / 11.10	—[b]	—	
V	H	H	70	>200 (dec) (C)	$C_7H_7ClKNO_3S$	5.38 / 5.39	12.85 / 12.32	—	
VI	H	H	50	161...162 (A)	$C_7H_8ClNO_5S$	5.71 / 5.51	12.61 / 12.63	13.93 / 13.97	
VIII (L-)	H	H	50	208...209 (dec.; B)	$C_8H_{11}ClN_2O_3S$	10.81 / 11.17	12.99 / 12.78	14.07 / 14.14	-10.5 (c = 1.04; nHCl)
IX (D-)	Me	Me	62	178...179 (dec.; B)	$C_{10}H_{15}ClN_2O_3S$	10.01 / 10.05	11.22 / 11.50	12.67 / 12.71	-52.5 (c = 0.99; nHCl)
X (L-)	H	H	84	153...154 (dec.; B)	$C_9H_{12}ClN_3O_4S$	14.21 / 14.26	11.00 / 10.86	11.98 / 12.03	-41.5 (c = 1.04; H_2O)
XI (D-)	Me	Me	37	156...157 (dec.; B)	$C_{11}H_{16}ClN_3O_4S$	13.00 / 13.05	10.04 / 9.96	10.88 / 11.01	-44.6 (c = 0.47; H_2O)

[a]Crystallization solvents: A(EtoH; (B) H_2O; (C)-ether-petroleum ether; (D) EtoAc.
[b]Found, %: C 52.62; H 5.03. Calculated, %: C 52.37; H 4.79.

Scheme 2


I
XVIc
XIII
XIV
a) R = ; R¹ = H
b) R = ; R¹ = H
c) R = ; R¹ = CHPh₂
XVa,b
A
94 %
CF₃COOH
Anisol
−4 °C
B
1) EtOH – H₂O
2) H⁺
III – XIIIb
XVIIb

Scheme 3

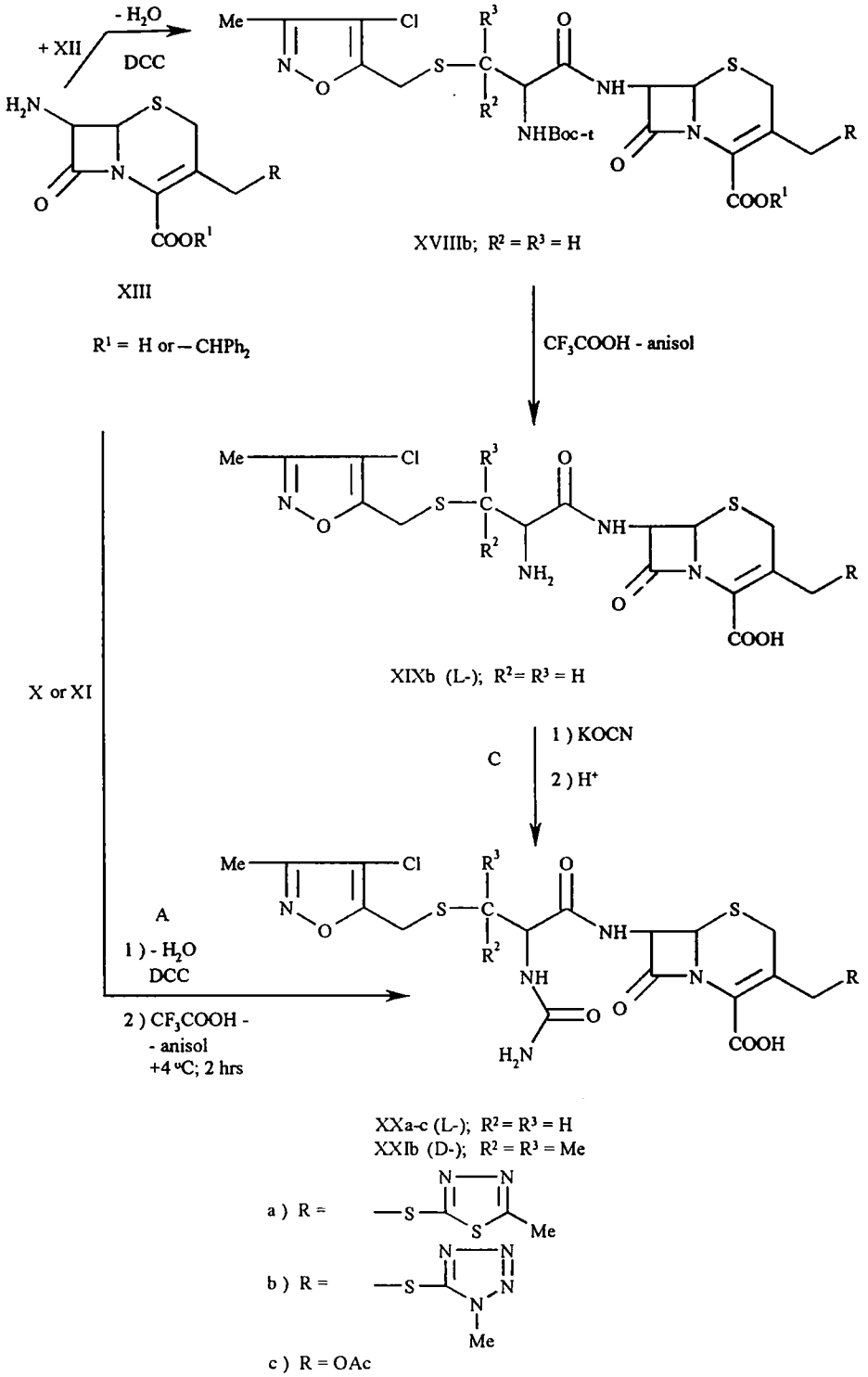

- H2O
DCC
+ XII
Me
Cl
R3
O
S
C
R2
NH
NHBoc-t
N
O
R
COOR1
H2N
XIII
R1 = H or – CHPh2
XVIIIb; R2 = R3 = H
CF3COOH - anisol
NH2
COOH
XIXb (L-); R2= R3 = H
X or XI
C
1) KOCN
2) H+
A
1) - H2O
DCC
2) CF3COOH -
- anisol
+4 °C; 2 hrs
XXa-c (L-); R2 = R3 = H
XXIb (D-); R2 = R3 = Me
a) R =
Me
b) R =
c) R = OAc

TABLE 3. Physicochemical Properties, IR- amd ^{1}H NMR-Spectroscopic Data of the New Cephalosporins

Compound	Yield, %	Mp, °C (cryst. solvent)	Molecular formula	Found, % / Calculated, %			IR-spectrum (KBr), cm^{-1}	^{1}H NMR (200 MHz, DMSO-d_6) δ, ppm
				Cl	N	S		
1	2	3	4	5	6	7	8	9
XVa	57.2	115 decomp. (EtOAc)	$C_{18}H_{17}ClKN_5O_5S_4$	6.12 / 6.04	11.71 / 11.95	22.07 / 21.88	2900...3000 (ν, CH_3); 1775 ($\nu_{C=O}$ β-lactam); 1680 (amide I); 1610 ($\nu_{C=N}$ isoxazole); 1520, 1380 (thiadiazole)	2.23 (3H, s, C–CH_3); 2.67 (3H, s, C–CH_3); 3.30 (2H, br s, $-CH_2-$); 3.42...3.74 (2H, AB_2, $-CH_2-$); 4.04 (2H, br s, $-CH_2-$); 4.23...4.54 (2H, AB_2, $-CH_2$); 5.04 (1H, d, –S–CH=N); 5.60 (1H dd, *CH*–N); 9.05 (1H, d, NH)
XVb	65	>145 decomp. (ether)	$C_{17}H_{17}ClKN_7O_5S_3$	6.27 / 6.21	16.58 / 17.19	16.80 / 16.29	3600...3100 (ν_{NH}); 3100...2900 ν_{CH_2}, CH_3); 1775 ($\nu_{C=O}$ β-lactam); 1675 (amide I); 1610; 1410 (isoxazole and CO_2^-)	* 2.31 (3H, s, C–CH3); 4.08 (3H, s, N–CH_3); 5.10 (1H, d, H-6); 5,55 (1H, d, H-7)
XVIc	92	84 decomp.	$C_{30}H_{29}N_7O_5S_2$	–	20.72 / 21.06	13.32 / 13.77	1782 ($\nu_{C=O}$, β-lactam); 1740 ($\nu_{C=O}$ ester); 1686 (amide I); 1514 (amide II); 1421 (isoxazole)	** 2.25 (3H, s, CH_3); 2.38 (3H, s, CH_3); 3.37 (2H, s, $-CH_2$-isoxazole); 3.76 (2H, s, 2-CH_2); 3.87 (3H, s, N–CH_3); 4.26...4.50 (2H, m, S–CH_2); 5.00 (1H, d, H-6); 5.87 (1H, d, H-7); 6.21 (1H, d, NH); 6.92 (1H, s, CH–Ar); 7.25...7.50 (10H, m, arom.)
XVIIb	79 (A) 31 (B)	189 decomp. (ether)	$C_{17}H_{19}N_7O_5S_2$	–	–	17.04 / 17.14	3271 (ν_{NH}); 2924 ($\nu_{CH_3CH_2}$); 1771 ($\nu_{C=O}$ β-lactam); 1708 ($\nu_{C=O}$ COOH); 1658 (amide I); 1530 (amide II); 1420 (isoxazole); 1232 (tetrazole)	2.15 (3H, s, CH_3); 2.37 (3H, s, CH_3); 3.35 (2H, s, $-CH_2$-isox.); 3.75 (2H, m, 2-SCH_2); 4.32 (2H, m, S–CH_2); 5.10 (1H, d, H-6); 5.70 (1H, dd, H-7); 9.11 (1H, d, NH); 13.67 (1H, br, COOH)

TABLE 3 (continued)

1	2	3	4	5	6	7	8	9
XIXb	47.2	167 decomp. (H_2O)	$C_{18}H_{21}ClN_8O_5S_3$	5.47 / 5.71	15.60 / 15.81	20.52 / 20.68	3400...3100 (ν_{OH}, ν_{NH}); 2930 (ν_s $-CH_2-$, CH_3-); 1776 ($\nu_{C=O}$ β-lactam); 1676 (amide I, amino acid); 1620 (ν_{as} CO_2^-); 1520 (amide II); 1413 (ν_s CO_2^-)	
XXa (L-)	77.2 (A) 38 (C)	149...150 decomp. (H_2O)	$C_{20}H_{22}ClN_7O_6S_4$	—	20.79 / 20.86	15.73 / 15.92	3365 (br OH, NH and NH_2); 1774 ($\nu_{C=O}$ β-lactam); 1660 (amide I); 1532 (amide II); 1409 (isoxazole)	2.25 (3H, s, CH_3-isoxazole); 2.58 (3H, s, CH_3-thiadiazole); 2,8...2,9 (ABX, AB, 2H, $-S-CH_2-CH=NH$); 3.57, 3.80 (AB, $-S-CH_2$, C-2); 3.95 (2H, s, $-CH_2-S$, isoxazole); 4.32, 4.55 (AB, 2H, $-CH_2-$ 17.02.98-S-thiadiazole); 4.50 (1H, s, br, CH side chain); 5.13 (1H, d, H-6); 5.68 (1H, dd, H-7); 6.35 (1H, d, NH ureido); 9.05 (1H, d, CONH)
XXb (L-)	94.3	142...144 decomp. (ether)	$C_{19}H_{22}ClN_9O_6S_3$	—	20.79 / 20.86	15.73 / 15.92	3460...3450 ($\nu_{NH,NH2}$); 1783 ($\nu_{C=O}$ β-lactam); 1680 (amide I); 1535 (amide II); 1445 (isoxazole); 1230 (tetrazole)	2.20 (3H, s, CH_3-isoxazole); 2.70...2.90 (2H, m, $-S-CH_2$); 3.60...3.85 (AB, 2H, $-CH_2-$); 3.95 (3H, s, $N-CH_3$); 4,0 (2H, s, $-CH_2-S-$); 4.20...4.40 ABq, $2H-S-CH_2-$); 4.40...4.60 (1H, m, CH-side chain); 5.10 (1H, d, H-6); 5.60...5.80 (1H, m, H-7 and 2H, NH_2); 6.35 (1H, d, NH-ureido); 9.05 (1H, d, CONH)
XXc (L-)	95	105...106 decomp. (H_2O)	$C_{19}H_{22}ClN_5O_8S_2$	6.15 / 6.46	12.45 / 12.78	11.72 / 11.70	3445...3349 (ν_{OH}, $\nu_{NH,NH2}$); 1774 ($\nu_{C=O}$ β-lactam); 1660 (amide I); 1532 (amide II); 1409 (isoxazole)	2.05 (3H, s, $-COCH_3$); 2.25 (3H, s, CH_3-isoxazole); 2.75...2.90 (ABX-AB, 2H, $-S-CH_2-CH-NH$); 3.5, 3.55 (AB, 2H, $-S-CH_2-$); 3.97 (2H, s, $-CH_2-S-$ isoxazole); 4.52 (1H, m, $-CH-$side chain); 4.70, 5.02 (AB, 2H, $-CH_2-OAc$); 5.12 (1H, d, H-6); 5.68 (1H, dd, H-7); 6.27 (1H, d, NH-ureido); 9.03 (1H, d, CONH)
XXIb (D-)	73	155...160 decomp. (ether)	$C_{21}H_{26}ClN_9O_6S_3$	5.46 / 5.60	20.26 / 19.94	15.21 / 15.21	3373 ((NH, (OH); 2971; 2915 (ν_{as}, ν_s $-CH_2-$, CH_3); 1787 ($\nu_{C=O}$ β-lactam); 1667 (amide I); 1530 (amide II); 1444 (δ_{CH3}); 1370 (δ_{CH3}); 1234 (tetrazole)	1.20 (3H, s, $C-CH_3$); 1.40 (3H, s, CH_3-C); 2.25 (3H, s, CH_3-isoxazole); 3.60; 3.76 (2H, AB, $-CH_2-$ dihydrothiazine); 3.93 (3H, s, $N-CH_3$); 3.94, 4.06 (2H, AB, -isoxazole-CH_2-S-); 4.24, 4.37 (2H, AB, $-CH_2-S-$tetrazole); 4.65 (1H, d, $CH-CO$); 5.10 (1H, d, H-6); 5.72 (3H, br, H-7+NH_2); 6.48 (1H, d, NH-ureido); 9.35 (1H, d, CONH)

Note. A, B and C method of synthesis; * in D_2O; **in $CDCl_3$.

TABLE 4. *In Vitro* Antibacterial Activity of the New Cephalosporins and Reference Compounds

Compound	MIC, μg/ml																			
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
XVa	>100	50	>100	>100	>100	>100	1.6	>100	>100	25	50	0.006	6.2	0.1	0.025	0.016	12.5	0.006	0.005	0.016
XVb	>100	25	>100	50	50	50	1.6	>100	>100	25	50	0.015	0.8	0.125	0.025	0.025	50	0.015	0.025	0.015
XVIIb	>100	3.1	50	50	6.2	6.2	0.4	>100	>100	3.1	3.1	0.006	1.6	0.20	0.05	0.05	50	0.015	0.015	0.015
XXa	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	3.1	50	12.5	6.2	3.1	>100	6.2	3.1	6.2
XXb	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	12.5	50	12.5	12.5	6.2	>100	12.5	6.2	6.2
XXc	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	3.1	100	25	12.5	12.5	>100	>12.5	6.2	>12.5
XXIb	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	1.6	>100	12.5	12.5	6.2	>100	6.2	3.1	12.5
Cefadroxil	>100	12.5	12.5	>100	12.5	12.5	6.2	>100	>100	6.2	12.5	0.4	50	3.1	0.8	0.8	>100	0.4	0.4	0.16
Cefuroxim	>100	3.1	12.5	12.5	3.1	6.2	0.01	12.5	25	3.1	3.1	0.05	6.2	1.6	0.8	0.8	>100	0.01	0.006	0.006
Cefamandol	50	0.4	50	>100	0.8	0.8	0.1	1.6	>100	1.6	0.8	0.05	3.1	0.8	1.6	0.1	50	0.05	0.05	0.05
Cefotaxim	>100	0.025	0.1	0.4	0.025	0.1	0.1	0.1	12.5	0.1	0.025	0.5	6.2	3.1	1.6	1.6	>100	0.006	0.006	0.01

1. *Bordetella bronchiseptica*. 2. *Escherichia coli* K12. 3. *Escherichia coli* 6 R*. 4. *Escherichia coli polyresist**. 5. *Escherichia coli* R-222*.
6. *Escherichia coli* R-15*. 7. *Klebsiella* ATCC 10031. 8. *Proteus vulgaris* XL. 9. *Pseudomonas aeruginosa*. 10. *Salmonella typhi-murium*. 11. *Shigella sonnei*.
12. *Bacillus* ATCC 6633. 13. *Staphylococcus aureus* 1110*. 14. *Staphylococcus aureus* 53.* 15. *Staphylococcus aureus* Smith. 16. *Staphylococcus epiderm* ATCC I-12228.
17. *Staphylococcus faecalis*. 18. *Staphylococcus haemolyticus* A-117. 19. *Streptococcus haemolyticus* A-118. 20. *Streptococcus haemolyticus* Robb.

*Beta-lactamase producer.

TABLE 5. Protective Activity of Acid XVIIb and Other Cephalosporins Against Systemic Infections in Mice (ED_{50}, mg/kg, 1 × s.c. and ED_{50}, mg/kg, 2 × p. O.)

Microorganisms inducing infection	Mode of administr.	ED_{50}, mg/kg			
		XVIIb	Cefuroxim	Cefamandol	Cefotaxim
Staphylococcus aureus Smith*	1 × s. c.	0.75	0.80	0.55	1.10
	2 × p. o.	15.0	13.5	6.2	100.0
Proteus vulgaris XL*	1 × s. c.	35.0	22.0	2.0	0.1
	2 × p. o.	>100.0	60.0	N. D.	N. D.
*Salmonella typhi-murium**	1 × s. c.	>100.0	5.0	5.8	1.4
	2 × p. o.	>100.0	—	N. D.	N. D.

*Beta-lactamase producer N. D. = not tested. Compound were administered 1 h.

TABLE 6. Serum Concentration of Acid XVIIB (mg/liter) in Mice After 20 mg/kg s.c.*

Time after dosing minutes	mg/l	Time after dosing minutes	mg/l	Time after dosing minutes	mg/l
30	2.15 (1.6 - 3.15)	60	0.48 (0 - 1.0)	120	0

*Minimum detectable concentration = 0.6 mg/liter (microbiological micro-agar-diffusion method using *Bacillus subtilis* ATCC.6633 as indicator organism).

Treatment of ester XIIIc with acid I in dichloromethane in the presence of N,N-dicyclohexylcarbodiimide (DCC) (20°C; 21 h) gave 92% of the benzhydryl ester XVIc. Removal of the ester group in a trifluoroacetic acid-anisol mixture (+4°C; 1.5 h) afforded 7-β-[(3,5-dimethylisoxazol-4-yl-acetamido)-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (XVIIb) (Method A). This new semi-synthetic antibiotic was found to be identical with that obtained by the reaction of XIIIb with the active ester III (20°C; 60 h) (Method B). However, the yield of this latter procedure (31%) was much lower than that of Method A.

Acylation of amino acid XIIIb with XII in dichloromethane in the presence of DCC (20°C; 20 h) led to XVIIIb, whose *t*-butyloxycarbonyl and benzhydryl protecting groups were removed simultaneously with trifluoroacetic acid-anisol (20°C; 2 h), and the resulting XIXb (L-) was isolated as a hygroscopic substance by freeze-drying from a pH ~4-5 aqueous solution (Scheme 3).

It is well-known that the structure of the acyl side chain significantly influences the antibacterial spectrum of β-lactam antibiotics. Earlier studies [17] showed that introduction of a dithiocarbamoyl group to the side chain resulted in antifungal cephalosporins with *in vitro* activity. In the case of the 7-ureidoacetyl cephalosporins the L-diastereoisomers were found to be surprisingly more effective against Gram-negative bacteria than the corresponding D-isomers [18, 19].

With this in mind we decided to synthesize semi-synthetic cephalosporins carrying an α-ureido function. Of the possible methods, application of that leading through a 2-aminooxazolone derivative [18] failed. In contrast, acylation of esters XIII (R^1 = $CHPh_2$) with the α-ureido acids X (L-) and XI (D-), described above, according to Method A, and subsequent removal of the protecting groups afforded the new cephalosporins XXa-c (L-) and XXIb (D-), respectively. The hydrolysis of the ester group was demonstrated by the IR-spectral data, i. e. by the disappearance of the ester band (1760-1730 cm^{-1}), and also the triple band-system (750, 740, and 690 cm^{-1}) characteristic of mono-substituted benzene rings. The successful deesterification was also indicated by the ^{1}H-NMR spectra, showing the lack of the CH proton (6.90 ppm) and of the 10 aromatic protons (7.20-7.70 ppm).

Compound XXb (L-) was also prepared, although in a moderate yield (38%), according to Method C by treatment of XIXb with potassium cyanate in aqueous medium (20°C; 3 h).

The most important and characteristic physical, IR-spectral, and ^{1}H-NMR-spectral data of the new cephalosporins synthesized according to Schemes 2 and 3 are collected in Table 3.

ANTIBACTERIAL ACTIVITY

In vitro Activity [20, 21]

The *in vitro* antibacterial activity of the synthesized cephalosporins in comparison with a few antibiotic preparations of the second and the third generation is demonstrated in Table 4.

The strains, which were originally clinical isolates, came from our collection. Some strains were beta-lactamase producers (marked with asterisk*). MIC values were assayed by microdilution method. Serial two-fold dilutions were used. In the Penassy Broth (2.9 ml in every tube) the inoculum was 10^4-10^5 CFU/ml. The medium was supplemented with 7.5% blood for *Streptococci*. The growth reactions were recorded after 24 h incubation at 37°C.

Of the synthesized compounds XVIIb was found to be the most active against test organisms 1-20. For strains 2, 10, and 11 XVIIb was 100% more effective than *Cefadroxil*, and possessed the same activity as *Cefuroxim*. The antibacterial activity of XVa and XVIIb against the Gram-positive strains 12-20 was almost one order of magnitude higher than that of the II and III generation preparations used in the comparative studies.

In contrast to previous reports [18, 19], the MIC values obtained for the L-α-ureido compounds XIXa-c were not significantly different from those of the D-isomer XXIb.

In vivo Activity

The remarkable *in vitro* antibacterial effect of XVIIb prompted us to carry out *in vivo* experiments, as well.

The protective effect of the compounds against experimental systemic infection was investigated in albino mice. The challenge CFU range was 10 to 20 times the number of bacteria required to kill 50% of the unmedicated control animals within 48 h. The 50% effective dose of antibiotics (ED_{50} mg/kg) was calculated by the probit method from the survived rates at the various (5 doses/compound) doses on the 5th day after infection.

Table 5 shows the protective activity of XVIIb in comparison with another cephalosporins against systemic infections on mice (ED_{50} values). Thus, compound XVIIb is active against the β-lactamase producing *Staphylococcus aureus* strain, but no therapeutic effect was observed in the case of *Proteus vulgaris* and *Salmonella typhi*. The results of the studies of the resorption experiments with XVIIb are shown in Table 6. The obtained serum concentration values indicate that this new semi-synthetic cephalosporin may be a candidate for the development of a drug-preparation for specific purposes.

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