SEMISYNTHETIC β-LACTAM ANTIBIOTICS. III[1,2] CEPHALOSPORIN DERIVATIVES IN THE FURYL SERIES

CHEMICAL AND MICROBIOLOGICAL PROPERTIES

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The synthesis of a series of 7-acylamido cephalosporins having a substituted furyl moiety in the side chain is described. These new cephalosporins were examined *in vitro* for antibacterial activity and evaluated for resistance to inactivation by β-lactamases.

In the endeavour to find cephalosporin antibiotics exhibiting a broad antibacterial activity, we prepared several 7-heterocyclic cephalosporins. Among the most promising derivatives were those wherein a furyl moiety in 7-side chain was present. In this series, consideration was given to the effect on activity of the position in the side chain of the sulphur atom which was attached, either directly or by a carbonyl group or by a methylene group, to the ring system.

The influence of the introduction of polar substituents in the furan ring and the introduction of a methyl group in the side chain was also examined. Microbiological screening enabled us to establish a spectrum and antibacterial activity of a certain importance for some of these molecules. Their sensitivity to cephalosporinase, compared with that of cephaloridine as the reference antibiotic, demonstrated that in some cases there is a considerable resistance to enzymatic hydrolysis by cephalosporinase extracted from Gram-negative organisms.

Chemistry

The new furylacylamido cephalosporins were synthesized from 7-ACA or 7-ADCA (**I**) through the intermediate bromo derivatives (**II**) ($R'=H$[3,4], $R'=CH_3$)[5] *via* nucleophilic displacement of the bromine atom with some appropriate thiols and thiocarboxylic acids. This reaction was carried out in aqueous acetone or dichloromethane; sodium bicarbonate or triethylamine was used respectively as acid acceptor.

Derivatives with R, R′, R″ are listed in Table 1. These compounds were isolated in crystalline form and their structures were characterized by spectra and analytical data; the NMR spectrum of each compound was consistent with the proposed structures. The chemical shifts of the protons of the cephalosporanic and desacetoxy cephalosporanic acids were in agreement with those described for 7-ADCA and 7-ACA[6] and not given in Table 1.

Antibacterial Activity

Fresh dilutions of the compounds were prepared daily by dissolving them in a saturated sodium bicarbonate solution directly or after first dissolving them in N-N-dimethylformamide when the antibiotics were only slightly soluble in the former solution.

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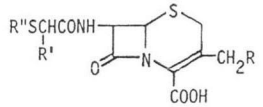
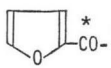
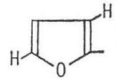
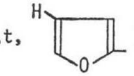
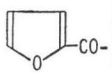
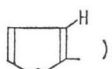
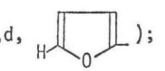
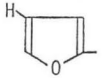
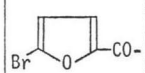
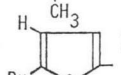
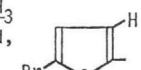
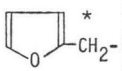
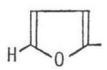
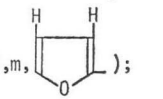
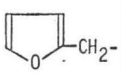
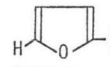
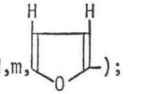
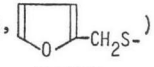
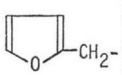
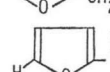
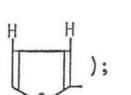
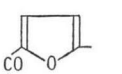
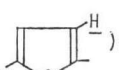
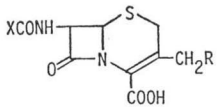
Table 1.

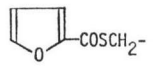
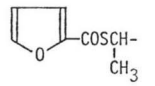
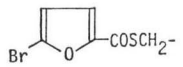
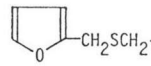
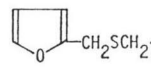
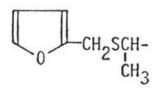
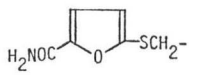


Compounds	R	R'	R"	Empirical formula	m.p. (°C)	Solvent of crystallization	NMR (δ)
1	$-OCOCH_3$	-H		$C_{17}H_{15}N_2O_8S_2Na$	169∿170	MeOH-Et_2O-hexane	9.30[a] (1H,d,-NHCO-); 8.03(2H,m, ); 7.30(1H,t, ); 3.93(2H,s,-SCH_2CO-)
2	$-OCOCH_3$	$-CH_3$		$C_{18}H_{18}N_2O_8S_2$	95∿96	CH_2Cl_2-CCl_4	9.33[a](1H,d,-NHCO-); 8.07(1H,d, ); 7.43(1H,d, ); 6.77(1H,m, ); 4.23(1H,m,-CH(CH3)-); 1.50(3H,d,-CH(CH3)-)
3	-H	-H		$C_{15}H_{16}BrN_2O_6S_2$	150∿151	MeCOMe-Light petr.	9.20[a](1H,d,-NHCO-); 7.43(1H,d, ); 6.86(1H,d, ); 3.93(2H,s,-SCH_2CO-)
4	$-OCOCH_3$	-H		$C_{17}H_{17}N_2O_7S_2Na$	182∿185	MeOH-*i*-PrOH	9.00[a](1H,d,-NHCO-); 7.60(1H,d, ); 6.40(2H,m, ); 3.90(2H,s,-SCH_2CO-); 3.23(2H,s,-CH_2S-)
5	-H	-H		$C_{15}H_{16}N_2O_5S_2$	90∿95	AcOEt	8.96[a](1H,d,-NHCO-); 7.53(1H,d, ); 6.33(2H,m, ); 3.86(2H,s,-SCH_2CO-); 3.23(2H,s, )
6	$-OCOCH_3$	$-CH_3$		$C_{18}H_{20}N_2O_7S_2$	89∿90	CH_2Cl_2-CCl_4	8.46[a](1H,d,-NHCO-); 7.37(1H,d, ); 6.27(2H,m, ); 4.50(1H,m,-CH(CH3)-); 3.83(2H,s,-CH_2S-); 1.50(3H,d,-CH(CH3)-)
7	$-OCOCH_3$	-H		$C_{17}H_{17}N_3O_8S_2$	150∿152	AcOEt-Et_2O-Light petr.	9.38[a](3H,m,-CO(NH2)-,-NHCO-); 7.33(1H,d, ); 6.86(1H,d, ); 4.00(2H,s,-SCH_2CO-)

*) isolated as sodium salt: a) disappeared after D_2O exchange

Table 2. Susceptibility of 89 clinical isolated to 7 new cephalosporins.



Compounds	R	X	*Staphylococcus aureus* (19)[a]	*Enterococcus* spp. (6)	*Escherichia coli* (22)	*Salmonella* spp. (10)	*Klebsiella* spp. (10)	*Proteus mirabilis* (12)	*Pseudomonas aeruginosa* (10)
			0.39[b]	25	25	50	50	50	100
1	$-OCOCH_3$		10	2	14		6	6	9
2	$-OCOCH_3$		6					1	6
3	$-H$		8		5		2	2	
4	$-OCOCH_3$		11	1	7	5	2	9	5
5	$-H$		7		1	3	2	3	5
6	$-OCOCH_3$		3					2	7
7	$-OCOCH_3$		8		14	3	4	3	9

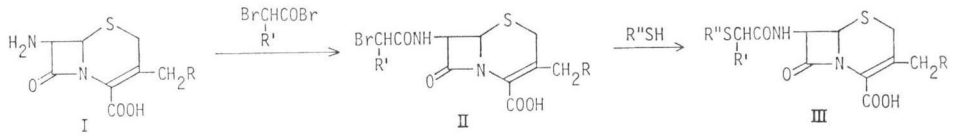
a) numbers in parenthesis indicate number of isolated strains
b) concentration (μg/ml) of the tested cephalosporins

Table 3. Hydrolysis (percent) of 7 new cephalosporins and cephaloridine (related antibiotic) by cephalosporinase extracted from 5 clinical isolates.

Cephalosporinase source	Rate of hydrolysis[a]							
	1	2	3	4	5	6	7	Cephaloridine
E. coli 1	50	—[b]	—	55	—	—	16	100
E. coli 19	14	—	—	22	—	—	10	100
E. freundii 2	36	72	125	30	134	84	25	100
P. rettgeri 26	18	—	—	30	—	—	12	100
P. aeruginosa 20	38	68	100	42	140	90	28	100

a) The rate of hydrolysis was determined at 37°C in 0.067 M potassium phosphate buffer (pH 7.0) with a substrate concentration of 50 μg/ml.
b) Not detected.

Scheme 1.



R=H, $-OCOCH_3$ R′=H, $-CH_3$ R″=see Table 1

Approximately 90 isolates of bacteria were employed. The organisms were obtained as clinical isolates from medical centres in Rome and identified by standard criteria (22 *Escherichia coli*, 10 *Salmonella* spp., 10 *Klebsiella* spp., 12 *Proteus mirabilis*, 10 *Pseudomonas aeruginosa*, 6 *Enterococcus* spp.) or taken from research laboratory cultures (19 penicillin-resistant and non-resistant *Staphylococcus aureus*).

Test cultures were grown to late log phase in nutrient broth with agitation at 37°C, diluted in fresh broth and dropped onto previously dried (4 hours, 37°C) nutrient agar plates so that the final inoculum size was approximately 10^6 organism/ml test medium (Penassay seed agar, Difco). The plates were incubated aerobically at 37°C.

The minimum inhibitory concentrations (MIC's) of the antibacterial agents, defined as the lowest drug concentration which completely prevents growth, were determined by a standard two-fold agar dilution method.

All studies were performed simultaneously in triplicate. Table 2 lists the more interesting results, expressed as the number of strains inhibited by more significant concentrations of new cephalosporins for each bacterial group.

Stability to β-Lactamases

(1) Preparation of β-lactamase:

The strains were grown at 37°C in Brain Heart Infusion broth (Difco). After overnight incubation the cells were harvested by centrifugation at 5,000×*g* for 30 minutes, washed once and suspended in 0.067 M phosphate buffer (pH 7.0). The cell suspensions were treated with an ultrasonic disintegrator (20 Kc/s for 2 minutes) with ice-cooling. After removal of cellular debris by centrifugation, the clear supernatant was subjected to gel filtration on a Sephadex G 100 column. The column was equilibrated with 0.067 M phosphate buffer (pH 7.0) and eluted with the same buffer. The enzyme-containing fractions were pooled and stored at −20°C.

(2) Assay of β-lactamase activity:

β-Lactamase activity was determined by the spectrophotometric method in a temperature controlled spectrophotometer at 30°C. The rate of hydrolysis of the β-lactam ring was followed by the change in UV-absorption at 260 nm with the substances at a concentration of 50 μg/ml. The relative initial rate of hydrolysis was expressed as a percentage of the rate of hydrolysis of cephaloridine.

Results

The minimum inhibitory concentrations of the cephalosporins discussed in this paper are listed in Table 2. The good activity of these compounds against Gram-positive microorganisms did not appear related to the position of the sulphur atom in the side chain, whereas the introduction of a methyl substituent in the α position of the sulphur atom gave less active compounds (**2** related to **1**; **6** related to **5**).

Some of the new cephalosporin derivatives proved to be fairly active against the tested Gram-negative microorganisms. In particular it is worth noting that **1** and **7** showed an inhibition of 64% of *E. coli* at a concentration of 25 μg/ml; **4** was active at a concentration of 50 μg/ml against 50% of *Salmonella* spp. and 75% of *P. mirabilis* while 90% of *P. aeruginosa* were susceptible to compound **1** at concentration of 100 μg/ml.

Furthermore, the results obtained with the cephalosporinase assays (Table 3) show that **1**, **4** and **7** were more resistant than cephaloridine against all 5 enzymes tested.

In conclusion, the more active compounds in the furyl series under examination appear to be derivatives **1** and **7** which exhibit a good antibacterial activity and marked cephalosporinase resistance, but no structure-activity relationships were found.

Experimental Section

All melting points were determined in open capillary tubes, using a Büchi SMP-20 apparatus.

NMR spectra were obtained in DMSO-d_6 (unless otherwise indicated) on a Varian T-60 spectrophotometer. Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within $\pm 0.4\%$ of theoretical values. Solvent evaporations were performed under reduced pressure below 40°C. Na_2SO_4 was used as the drying agent for organic extracts. The yields were between 45% and 75%.

Preparation of 5-bromo, thio-2-furoic acid

To a solution of 5-bromo, 2-furoic acid (2 g, 0.01 mol) in benzene (15 ml), thionyl chloride (8 ml, 0.11 mol) was added and the resulting solution was allowed to reflux for 2 hours.

The mixture was evaporated *in vacuo*. The residual crude 5-bromo, 2-furoic acid chloride was added dropwise to a saturated solution of $NaHS \cdot xH_2O$ (4.5 g) in ethanol 95% (15 ml).

The mixture was stirred at 15°C for 1 hour. The precipitated sodium chloride was quickly filtered off. The filtered solution was evaporated to dryness under reduced pressure to give crude sodium salt of the title thioacid, which was taken up with cold water (10 ml). The pH was adjusted to 2 with conc. HCl and the solution extracted with ethyl ether. The organic layer was washed with water, then dried and evaporated *in vacuo* to give crude 5-bromo, thio-2-furoic acid which was directly used for the next reaction.

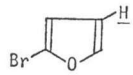
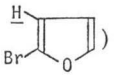
Preparation of 7-[5-bromo-thio-2-furoylacetamido]-desacetoxy cephalosporanic acid (**3**, Table 1)

To a vigorously stirred solution of 7-bromoacetamido desacetoxy cephalosporanic acid (3.0 g, 0.09 mol) in acetone (15 ml) and 0.4 N $NaHCO_3$ (50 ml), 5 bromo, thio-2-furoic acid (1.81 g, 0.09 mol) in acetone (15 ml) was added. After 48 hours at room temperature, the solution was concentrated *in vacuo* at 30°C to eliminate acetone then the pH was adjusted to 3 with conc. HCl and the solution was extracted with ethyl acetate.

The organic layer was separated, dried and concentrated *in vacuo* to give crude product which was crystallized from ethyl acetate - ethyl ether - light petroleum, m.p. 150~151°C.

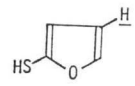
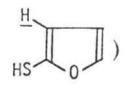
Anal. ($C_{15}H_{13}BrN_2O_6S_2$) C, H, Br, N, S.

Preparation of 2-mercapto, 5-furan-carboxamide

2-Bromo, 5-furoic acid (1.91 g, 0.01 mol) and thionyl chloride (8 ml, 0.11 mol) were heated reflux for 2 hours. Then volatile material was removed *in vacuo* and washed with anhydrous ethyl ether. The residual crude acid chloride was added dropwise to NH_4OH (15 ml) at 0°C. The solution was stirred for 2 hours, then concentrated *in vacuo*. The crude product was washed with H_2O, filtered and dried to give pure 2-bromo, 5-furan-carboxamide. m.p. 155~156°C, yield 80%, NMR (CD_3COCD_3) δ 7.23~7.07 (3H, m, NH_2CO- and ); 6.77 (1H, d ) Anal. ($C_5H_4BrNO_2$)C, H, Br, N.

To a solution of $Na_2S \cdot 9H_2O$ (5.6 g, 0.023 mol) in water (10 ml) was added 2-bromo, 5-furan-carboxamide (1.5 g, 0.007 mol) in ethanol (15 ml). The solution was stirred at 45°C for 24 hours, then concentrated *in vacuo*, acidified to pH 3 and extracted with ethyl acetate. The organic layers were dried and concentrated *in vacuo*.

The residue was dissolved in saturated solution of $NaHCO_3$ then the pH was adjusted to 3 with conc. HCl at 0°C. The resulting precipitate was collected by filtration and dried. m.p. 183~185°C, yield 61%. NMR (DMSO)

δ 7.20 (1H, d, ); 6.73~6.40 (3H, m, NH_2CO- and )

Anal. ($C_6H_5NO_2S$) C, H, N, S.

Preparation of 7-[S-(5-carboxamido, 2-furyl)-mercaptoacetamido]cephalosporanic acid (**7**, Table 1)

To a stirred solution of 7-bromoacetamido cephalosporanic acid (2.75 g, 0.007 mol), chloroform anhydrous (30 ml) and triethylamine (0.9 ml, 0.007 mol), 2-mercapto,5-furancarboxamide (1.00 g, 0.007 mol), dissolved in acetone (10 ml) and chloroform (50 ml), was added; the solution was stirred for 72 hours, then concentrated *in vacuo*. The residual crude product was taken up with saturated solution of $NaHCO_3$ and the pH was adjusted to 3 with conc. HCl. The resulting precipitate was collected by filtration and dried.

Recrystallization of the crude solid from ethyl acetate - ethyl ether - light petroleum gave pure compound, m.p. 150~152°C.

Anal. ($C_{17}H_{17}N_3O_8S_2$) C, H, N, S.

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