SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF PYRIMIDINYLUREIDOCEPHALOSPORINS

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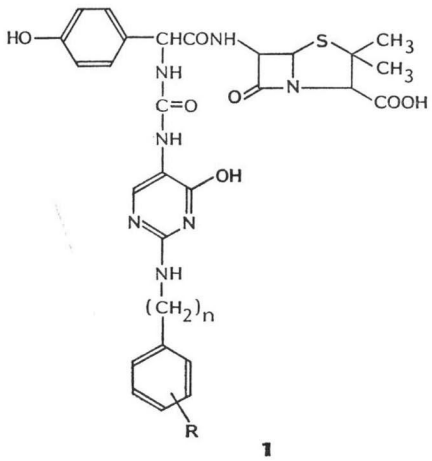
The synthesis of a series of 7*R*-[(*R*)-2-[3-[5-pyrimidinyl]ureido]-2-(aryl)acetamido]-3-cephem-4-carboxylates is described. Variation of the substituents at the 3-position in the cephem nucleus, at the 2-position of the pyrimidine ring, and of the phenyl residue in the acyl side chain is carried out. Qualitative structure-activity relationships in this series are discussed. VX-VD 2, the most interesting compound, exhibits broad antimicrobial activity against Gram-negative bacteria, including *Pseudomonas aeruginosa*.

Infections caused by Gram-negative bacteria have become a serious problem in clinical practice. This situation has initiated a search for β-lactam antibiotics active against a wide range of Gram-positive and Gram-negative bacteria, including species of *Escherichia coli*, *Klebsiella*, *Proteus*, *Serratia*, *Enterobacter* and *Pseudomonas*.

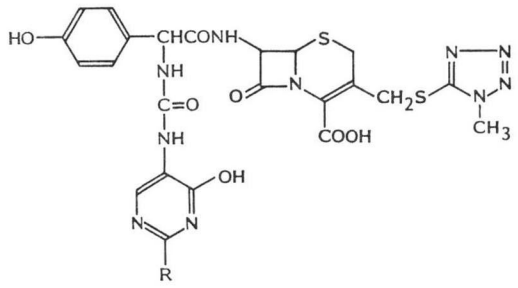
In our previous work we investigated the pyrimidinylureidopenicillins **1**, a new class of penicillins with broad spectrum activity[1].

Fig. 1.



1

Fig. 2.

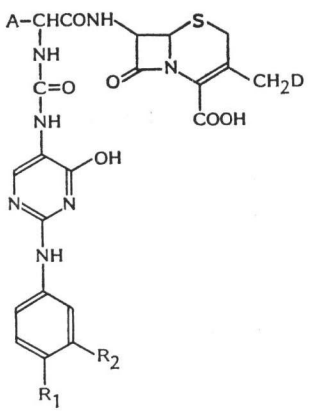


2

The promising antibacterial spectrum of these compounds, especially their remarkable activity against *Pseudomonas aeruginosa*, prompted us to synthesize the analogues in the cephalosporin series. Our main interest in this field was also directed towards compounds active against *Pseudomonas*, a property missing in most of the broad spectrum cephalosporins currently in therapeutic use.

Among a set of cephalosporins **2** with different substituents R, *e.g.* alkyl, aralkyl, alkylamino, dialkylamino, aralkylamino and anilino, in the pyrimidine ring, the anilino derivatives showed the highest activity[2]. This paper deals with the synthesis and the structure-activity relationships of the

Fig. 3.



8a ~ 8v

anilinocephalosporins **8**.

Chemistry

The cephalosporins **8** were mainly prepared by acylation of the diphenylmethyl 7-amino-3-cephem-4-carboxylates (**4**) with the mixed anhydride derived from the ureidocarboxylic acids (**3**)[1] and isobutyl chloroformate, followed by cleavage of the diphenylmethyl protecting group using TFA/anisole.

An alternative method — reaction of the amino group of compound **5** with 1-hydrooxazolo[5.4-d]pyrimidine-2-one (**6**)[1] — was developed for the preparation of **8g**.

The starting material **5** was prepared by condensation of diphenylmethyl 7-amino-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate with D-(*N*-*t*-butyloxycarbonyl)-4-hydroxyphenylglycine in the presence of *N*,*N*′-dicyclohexylcarbodiimide and subsequent removal of the protecting groups using TFA/anisole.

Antibacterial Activity

The cephalosporins **8** offer three possibilities for a structural modification; variation of A, D and the substituent pattern of the anilino group. Based on the sites of variation, Table 1, 2 or 3 respectively contain the MIC values of the cephalosporins **8** against *Staphylococcus aureus* and 6 Gram-negative bacterial strains.

Table 1 shows the effect of R_1 and R_2 on antimicrobial activity. Polar, electron withdrawing substituents R_1 lead to enhanced antimicrobial activity, a result similar to those found in the related penicillins **1**[1]. A second polar substituent R_2 does not further increase the antimicrobial activity.

The influence of the substituent D on antimicrobial activity is given in Table 2. Displacement of the *N*-methyltetrazolyl-5-thio group by other *N*-alkyltetrazolyl-5-thio residues (**8q**~**8s**), by the acetoxy

Fig. 4.

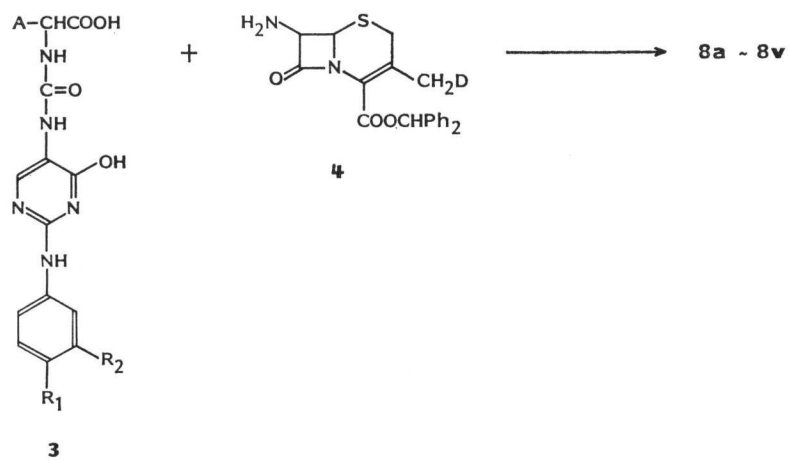


Fig. 5.

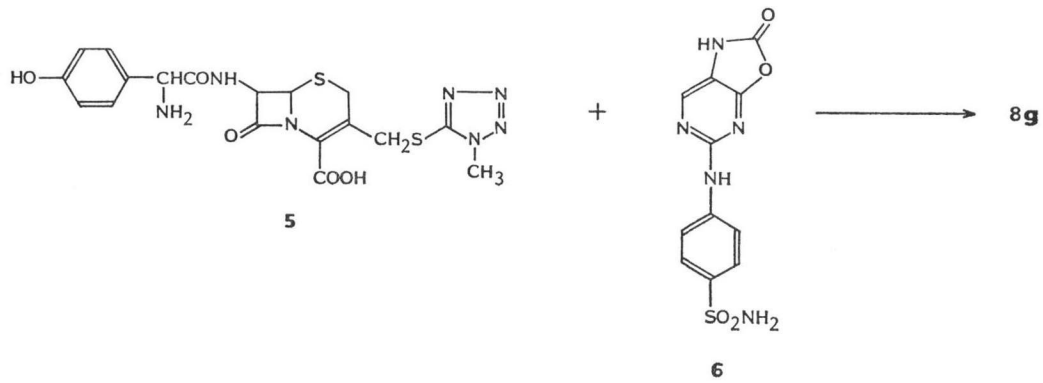


Table 1. Pyrimidinylureidocephalosporins **8** (A=4-hydroxyphenyl, D=*N*-methyltetrazolyl-5-thio).

8	R_1	R_2	MIC (μg/ml)[a]						
			S.a.	P.a.	K.p.	S.m.	E.c.	Eb.c.	P.r.
a	H	H	0.5	8	0.5	1	0.25	0.5	2
b	Cl	H	0.5	8	0.5	1	0.5	0.5	2
c	OH	H	1	8	0.5	1	0.25	0.5	2
d	$N(CH_3)_2$	H	1	8	0.5	1	0.5	0.5	2
e	$NHCONH_2$	H	1	8	1	1	0.25	2	2
f	$CONH_2$	H	0.5	4	0.25	0.5	0.06	0.25	1
g	SO_2NH_2	H	1	4	0.13	0.13	0.06	0.13	0.25
h	$SO_2N(CH_3)_2$	H	2	8	0.5	0.5	0.13	0.5	1
i	$CONH_2$	$CONH_2$	1	8	0.5	0.5	0.25	1	0.5
j	$CONH_2$	OH	0.5	8	0.25	0.25	0.13	0.25	0.5
k	SO_2NH_2	OH	1	8	0.25	0.25	0.13	>64	0.5

[a] The MIC's were determined by a serial dilution test with the factor 2 (meat bouillon extract, inoculum 3×10^5).
Test organisms and abbreviations: S.a., *Staphylococcus aureus* SG 511; P.a., *Pseudomonas aeruginosa* Hbg.; K.p., *Klebsiella pneumoniae* ATCC 10031; S.m., *Serratia marcescens* ATCC 13880; E.c., *Escherichia coli* ATCC 11775; Eb.c., *Enterobacter cloacae* ATCC 13047; P.r., *Proteus rettgeri* BC 7.

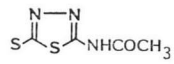
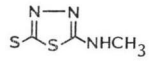
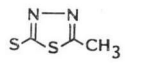
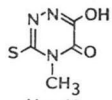
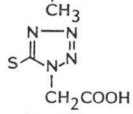
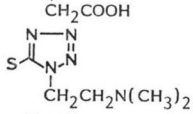
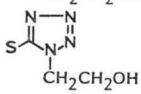
group (**8l**), or by other heterocyclic thio groups (**8m**~**8p**) leads to no higher activity against Gram-negative bacteria.

Replacement of the 4-hydroxyphenyl group in position A by phenyl, 3,4-dihydroxyphenyl or 2-aminothiazol-4-yl, as shown in Table 3, does not enhance the overall antimicrobial activity. The phenyl and the 2-aminothiazol-4-yl group (**8t**, **8v**) lead to reduced activity against *P. aeruginosa*. The 3,4-dihydroxyphenyl group (**8u**) provides equal potency against *P. aeruginosa*, but the activity against the other Gram-negative strains is diminished.

In tests against *Enterobacter* P99, *E. coli* 11775 R^+TEM and *Klebsiella pneumoniae* 1082E, carrying β-lactamase I, III and Vc respectively, none of the cephalosporins **8** showed appreciable activity.

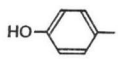
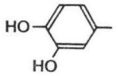
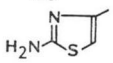
Tables 1~3 show **8g** to be the most promising compound in this series with a high activity against sensitive Gram-negative bacteria. In an *in vitro* comparison to cefotaxime and cefoperazone, compound **8g** proves more active against *P. aeruginosa* and *Enterobacter* than cefotaxime, and has similar activity as cefoperazone. First *in vivo* tests however showed **8g** to be superior to cefoperazone. In mice

Table 2. Pyrimidinylureidocephalosporins **8** (R_1=4-aminosulfonyl, R_2=H, A=4-hydroxyphenyl).

8	D	MIC (μg/ml)[a]						
		S.a.	P.a.	K.p.	S.m.	E.c.	Eb.c.	P.r.
g		1	4	0.13	0.13	0.06	0.13	0.25
l	$OCOCH_3$	1	4	0.5	1	0.25	4	0.5
m		2	8	1	2	0.5	1	2
n		2	8	0.25	0.5	0.25	2	1
o		1	8	0.5	0.5	0.25	8	0.5
p		0.13	8	0.13	0.25	0.13	0.25	0.25
q		8	8	0.5	0.5	0.5	2	0.25
r		4	64	1	1	0.25	1	2
s		1	4	0.5	0.13	0.13	0.25	0.25
Cefotaxime		1	16	0.06	0.25	0.03	1	0.015
Cefoperazone		1	4	0.13	0.25	0.03	0.13	0.5

[a] See the footnote in Table 1.

Table 3. Pyrimidinylureidocephalosporins **8** (R_1=4-aminosulfonyl, R_2=H, D=*N*-methyltetrazolyl-5-thio).

8	A	MIC (μg/ml)[a]						
		S.a.	P.a.	K.p.	S.m.	E.c.	Eb.c.	P.r.
g		1	4	0.13	0.13	0.06	0.13	0.25
t		0.25	8	0.13	0.25	0.13	0.13	0.5
u		16	4	2	4	4	8	2
v		1	16	0.06	0.25	0.13	0.25	0.13

[a] See the footnote in Table 1.

infected with *E. coli* ATCC 11775 or *K. pneumoniae* BC 6, **8g** given sc had ED_{50} values of 0.3 or 7.6 mg/kg and compared favorably with cefoperazone (ED_{50} values of 3.1 and 77.4 mg/kg). Therefore this compound, coded VX-VD 2, was selected for further investigation to be published later.

Experimental

Methods

IR spectra were measured in KBr disk using a Perkin-Elmer infrared spectrometer. 80 MHz spectra were recorded on a Bruker WP 80 instrument with TMS as internal standard. Characteristic synthetic procedures for starting materials and cephalosporins are outlined in the following examples. The structures of all cephalosporins were confirmed by analytical data.

Material

Compounds **3** were prepared following a procedure reported in ref 1. Diphenylmethyl 7-amino-3-[(heteroarylthio)methyl]-3-cephem-4-carboxylates (**4**) were prepared from 7-aminocephalosporanic acid by replacement of the acetoxy group with a heterocyclic thio group and subsequent esterification of the 4-carboxylic group with diphenyldiazomethane following known methods.

7*R*-[(*R*)-2-[3-[2-(4-Aminocarbonyl)anilino-4-hydroxy-5-pyrimidinyl]ureido]-2-(4-hydroxyphenyl)-acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic Acid, Sodium Salt (**8f**)

To a solution of (*R*)-2-[3-[2-(4-aminocarbonyl)anilino-4-hydroxy-5-pyrimidinyl]ureido]-2-(4-hydroxyphenyl)acetic acid (1.1 g, 2.5 mmol) in CH_2Cl_2 (10 ml) and DMF (20 ml) was added *N*-methylmorpholine (0.23 g, 2.53 mmol). After stirring at room temperature for 30 minutes, the mixture was chilled to −35°C. Isobutyl chloroformate (0.34 g, 2.53 mmol) was added and the temperature maintained at −35°C for 10 minutes.

A solution of diphenylmethyl 7-amino-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (1.24 g, 2.5 mmol) in CH_2Cl_2 (15 ml) and DMF (5 ml), cooled to −40°C, was added. The resulting mixture was stirred at −35°C for 30 minutes and then allowed to warm to room temperature within 2 hours.

The reaction solution was poured into an ice-cold mixture of H_2O (35 ml) and MeOAc (35 ml) and the aqueous layer was discarded. The organic layer was washed successively with 5% aqueous $NaHCO_3$ and with H_2O, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was treated with MeOH and the solid diphenylmethyl ester of **8f** filtered off.

A stirred suspension of the crude ester in CH_2Cl_2 (5 ml) and anisole (2 ml) was treated with TFA (5 ml) at 0°C for 30 minutes. The resulting solution was evaporated *in vacuo*, triturated with toluene, and evaporated.

The remaining cephalosporanic acid and an equimolar amount of sodium 2-ethyl hexanoate were dissolved in DMF.

8f (1.1 g, 57.1%) precipitated as an amorphous powder by addition of Et_2O. IR (KBr) 1760, 1660, 1600, 1510 cm^{-1}; NMR (DMSO-d_6 - CD_3OD) δ 3.55 (q, 2H), 3.95 (s, 3H), 4.3 (m, 2H), 4.9 (d, 1H), 5.45 (s, 1H), 5.65 (d, 1H), 6.75 (d, 2H), 7.35 (d, 2H), 7.65~8.1 (m, 4H), 8.35 (s, 1H).

D-(*N*-*t*-Butoxycarbonyl)-4-hydroxyphenylglycine

To a solution of D(−)-4-hydroxyphenylglycine (40 g, 0.24 mol) and triethylamine (24.5 g, 0.245 mol) in 50% aqueous dioxane (300 ml) di-*t*-butyldicarbonate (57.5 g, 0.26 mol) was added slowly at 20°C and the mixture was stirred for 15 hours. Dioxane was distilled off *in vacuo* at 30°C, ice-water was added and the mixture extracted three times with EtOAc.

The aqueous layer was cooled to 0~5°C, adjusted to pH 1.5 with 2 N HCl and extracted with EtOAc. The organic layer was washed with H_2O, and dried ($MgSO_4$). Petroleum ether was added, and the solution stored at 0~5°C overnight to give D-(*N*-BOC)-4-hydroxyphenylglycine (61.6 g, 96%); mp 121~122 °C; IR (KBr) 1735, 1675, 1550, 1515 cm^{-1}; NMR ($CDCl_3$ - CD_3OD) δ 1.4 (s, 9H), 5.0 (s, 1H), 6.75 (d, 2H), 7.2 (d, 2H).

Diphenylmethyl 7*R*-[(*R*)-2-(*t*-Butoxycarbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate

To an ice-cold solution of diphenylmethyl 7-amino-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (3.55 g, 7 mmol) in anhydrous THF (100 ml) was added successively a solution of D-(*N*-BOC)-4-hydroxyphenylglycine (2.27 g, 8.5 mmol) in anhydrous DMF (30 ml), and a solution

of dicyclohexylcarbodiimide (1.8 g, 8.7 mmol) in anhydrous THF (30 ml) at 0°C. After stirring for 5 hours at 0°C and 2 hours at room temperature the precipitated dicyclohexylurea was filtered off, and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (1,000 g) using CH_2Cl_2 - MeOH (30: 1) as the eluant to give diphenylmethyl 7*R*-[(*R*)-2-(*t*-butoxycarbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (3.1 g, 59.5%); IR (KBr) 1785, 1710, 1610, 1590 cm^{-1}; NMR ($CDCl_3$ - CD_3OD) δ 1.4 (s, 9H), 3.55 (m, 2H) 3.90 (s, 3H), 4.3 (m, 2H), 4.9 (d, 1H), 5.15 (s, 1H), 5.75 (d, 1H), 6.8 (d, 2H), 6.95 (s, 1H), 7.1~7.65 (m, 12H).

7*R*-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic Acid (**5**)

A solution of diphenylmethyl 7*R*-[(*R*)-2-(*t*-butoxycarbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylate (7.44 g, 10 mmol) in TFA (30 ml) and anisole (6 ml) was stirred at 0°C for 30 minutes. The reaction mixture was evaporated *in vacuo*, the residue treated with Et_2O, and filtered to give **5** (trifluoroacetic acid salt, 4.96 g, 84%), which was used in the subsequent step without further purification.

7*R*-[(*R*)-2-[3-[2-(4-Aminosulfonyl)anilino-4-hydroxy-5-pyrimidinyl]ureido]-2-(4-hydroxyphenyl)-acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic Acid, Sodium Salt (**8g**)

A solution of **5**, (trifluoroacetic acid salt, 5.9 g, 10 mmol) in 75% aqueous THF (200 ml) was adjusted to pH 7.5 by the addition of 1 N NaOH at 0~5°C. 1-Hydro-5-(4-aminosulfonyl)anilino-oxazolo[5.4-d]pyrimidine-2-one (3.1 g, 10 mmol) was added in portions with stirring, maintaining the pH value at 7.5 by means of 0.1 N NaOH. The mixture was stirred for 1 hour without cooling, H_2O (50 ml) was added and the THF distilled off *in vacuo*. The aqueous solution was extracted with EtOAc (3×50 ml), and acidified at 0°C with 2 N HCl to pH 3.0. The precipitate was collected, washed with H_2O - MeOH and dried.

Addition of Et_2O to an equimolar solution of this acid and sodium 2-ethylhexanoate in DMF gave **8g** as a colorless powder, yield 6.6 g (81%); IR (KBr) 1760, 1655, 1590, 1530 cm^{-1}; NMR (DMSO-d_6 - CD_3OD) δ 3.45 (m, 2H), 3.9 (s, 3H), 4.3 (m, 2H), 4.9 (d, 1H), 5.4 (s, 1H), 5.55 (d, 1H), 6.70 (d, 2H), 7.25 (d, 2H), 7.7 (d, 2H), 7.95 (d, 2H), 8.3 (s, 1H).

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