STUDIES ON SEMI-SYNTHETIC 7α-FORMAMIDOCEPHALOSPORINS

I. STRUCTURE-ACTIVITY RELATIONSHIPS IN SOME SEMI-SYNTHETIC 7α-FORMAMIDO-CEPHALOSPORINS

Sir:

The isolation[1] of 7α-methoxycephalosporins from *Streptomyces* species in 1971 stimulated considerable interest[2] in the synthesis of 7α(6α)-substituted cephalosporins and penicillins. However, other than the methoxy group, only few[3,4] 7α(6α)-substituents lead to antibacterially active compounds. One notable exception is the 7α(6α)-formamido group[5], discovered through a chemical programme[6] designed to identify β-lactamase stable antibiotics. Subsequently other workers have described the isolation and antibacterial properties of several naturally occurring 7α-formamidocephalosporins from *Flavobacterium*, *Lysobacter* and *Xanthomonas* species[7~12], and 3α-formamidonocardicin analogues from *Flexibacter aliginoliquefaciens*[13,14]. We have previously reported[15] the antibacterial activity of BRL 36650, a 6α-formamidopenicillin with exceptional activity against *Pseudomonas* species and against members of the family Enterobacteriaceae, and some preliminary structure-activity relationships of other 6α-formamidopenicillins have been published[6,16]. This communication describes a comparison of activity of some 7α-substituted cephalosporins with the corresponding 7α-formamido analogue, and then outlines some structure-activity relationships in a series of these formamidocephalosporins.

The synthesis of the 3-(acetoxymethyl)cephems is most conveniently carried out by direct acylation of the 7α-formamido nucleus[5] (**1**) with the appropriate activated side chain acid. Alternatively they may be prepared by a similar acylation of the 7α-(methylthio) nucleus (**2**) followed by amination (NH_3, $Hg(OAc)_2$, DMF, −40°C) and formylation, or through acylation of the 7α-formamidocephaloglycin ester (**4**). 3-[(Heterocyclyl)thiomethyl]cephems are obtained by coupling the nucleus (*e.g.*, **3**) with a suitable phenylglycine derivative. Standard deprotection conditions (TFA or TFA/anisole) then afford the desired 7α-formamidocephalosporins. A full account of the synthesis and chemistry of these compounds is to be published elsewhere[17].

The antibacterial activity of the 7α-formamidocefoperazone analogue (**8**) compared with other related 7α-substituted cephalosporin derivatives is shown in Table 1. The four compounds (**5~8**) were active against members of the family Enterobacteriaceae and, with the exception of the 7α-(hydroxymethyl) compound (**7**), showed some activity against Gram-positive bacteria. The advantage of the 7α-substituted cephalosporins (**6~8**) over the unsubstituted derivative (**5**) is their greater stability to bacterial β-lactamases. This may be seen by comparison of their activity against *Escherichia coli* DCO and the corresponding plasmid-mediated β-lactamase producing strain, *E. coli* DCO RTEM. The 7α-formamido substituent is particularly advantageous compared with 7α-methoxy and 7α-(hydroxymethyl) substituents as it confers greater intrinsic activity against Gram-negative bacteria, including *Pseudomonas aeruginosa*.

In an attempt to improve upon this activity, several other ureido and acylamino cephalosporins were prepared. The activity of some of these (**9~12**) is shown in Table 1. However, the 7α-formamidocefoperazone analogue (**8**) remained the most potent of the cephalosporins tested, being at least 2~4-fold more active than the other ureido or acylamino derivatives.

Alternative approaches to improve the activity of **8** included substitution at C-3 and derivatisations of the phenyl ring. It will be seen from Table 1 that introduction of a 3-[(*N*-methyltetrazolyl)thiomethyl] group results in a 2-fold increase in activity against all the bacteria tested (see compound **13**). This level of activity is maintained by introduction of a 4-hydroxy substituent into the phenyl ring (compound **14**). However, further substitutions, giving the 3,4-dihydroxy derivative (**15**), results in a pronounced increase in activity against Gram-negative organisms, albeit at the expense of some activity against Gram-positive bacteria. Overall, compound **15** possessed very potent activity particularly against strains of *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa*.

These results show that although the naturally occurring 7α-formamidocephalosporins have only poor antibacterial activity[7,8,12] there are semisynthetic derivatives which display outstanding broad-spectrum activity, with demonstrable advantages *in vitro* over the newer cepha-

Table 1. The relative antibacterial activities[a] *in vitro* of some 7α-formamido and 7α-substituted cephalosporins.

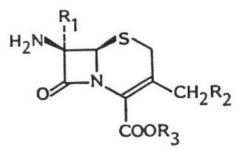
Organism	5	6	7	8	9	10	11	12	13	14	15	CPZ	CAZ
Escherichia coli NCTC 10418	0.25	0.25	2.0	0.12	4.0	0.12	2.0	2.0	0.06	0.06	≤0.03	≤0.06	0.12
E. coli DCO	2.0	2.0	2.0	0.25	16	—	—	—	0.12	0.12	≤0.03	≤0.06	0.12
E. coli DCO RTEM[b]	32	2.0	4.0	0.25	16	0.12	1.0	1.0	0.12	0.12	≤0.03	1.0	0.25
Enterobacter cloacae N1	4.0	8.0	8.0	1.0	64	16	4.0	16	0.5	0.5	0.12	2.0	0.25
Klebsiella pneumoniae T767	2.0	4.0	0.5	0.12	32	2.0	1.0	4.0	0.12	0.25	≤0.03	0.25	0.25
Proteus mirabilis C977	2.0	2.0	2.0	0.5	32	8.0	2.0	4.0	0.5	0.5	0.12	1.0	0.12
Serratia marcescens US32	16	64	4.0	1.0	64	8.0	4.0	8.0	0.25	0.25	0.12	2.0	0.5
Pseudomonas aeruginosa NCTC 10662	8.0	64	>128	8.0	64	0.5	32	32	4.0	4.0	0.25	4.0	1.0
P. aeruginosa Dalgleish[b]	64	128	>128	4.0	32	0.5	32	32	4.0	4.0	0.25	32	0.5
Staphylococcus aureus Oxford	1.0	8.0	>128	8.0	8.0	16	8.0	8.0	4.0	8.0	16	2.0	8.0
S. aureus Russell[b]	16	8.0	>128	8.0	8.0	16	4.0	8.0	4.0	8.0	16	16	16
Streptococcus pyogenes CN10	0.12	1.0	128	1.0	0.25	8.0	0.5	—	0.12	0.25	0.5	0.12	0.12

[a] MICs (μg/ml) determined by serial dilution in nutrient agar containing 5% defibrinated horse blood, inoculum 0.001 ml of an overnight broth culture.

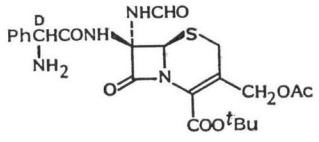
[b] Plasmid-mediated β-lactamase-producing strain.

CPZ: Cefoperazone.

CAZ: Ceftazidime.



1 R_1 = NHCHO R_2 = OAc R_3 = ^{t}Bu
2 R_1 = SCH_3 R_2 = OAc R_3 = ^{t}Bu
3 R_1 = NHCHO R_2 = STet R_3 = $CHPh_2$



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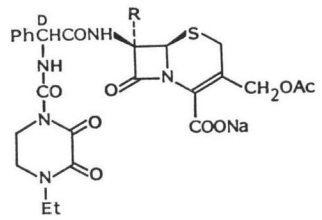
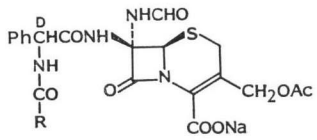
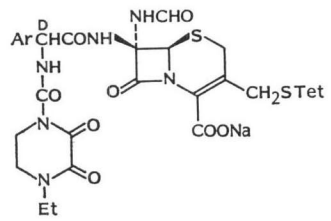


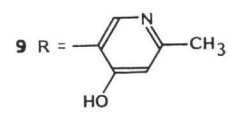
5 R = H **7** R = CH_2OH
6 R = OCH_3 **8** R = NHCHO



9 ~ 12



13 ~ 15



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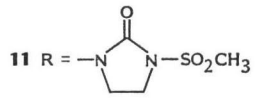

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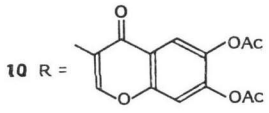

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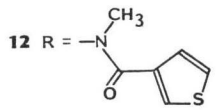
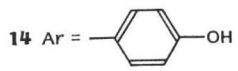


12 R =

13 Ar = Ph



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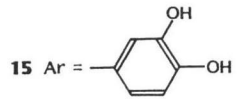

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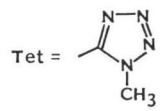

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losporins, cefoperazone and ceftazidime. Further modifications to optimise activity in this series are under investigation and will be the subject of further publications.

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