STUDIES ON SEMI-SYNTHETIC 7α-FORMAMIDOCEPHALOSPORINS

III. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME 7β-[D-2-(ARYL)-2-[(4-ETHYL-2,3-DIOXOPIPERAZIN-1-YL)-CARBONYLAMINO]ACETAMIDO]-7α-FORMAMIDO-CEPH-3-EM-4-CARBOXYLATE DERIVATIVES

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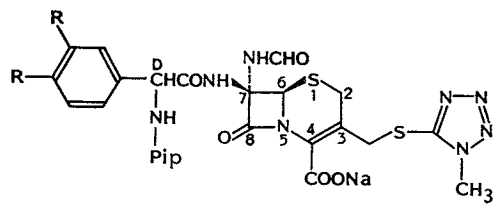
The synthesis and antibacterial activity of 7β-[D-2-(aryl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido]-7α-formamidocephalosporins with various substituents at the C-3 position of the cephalosporin nucleus is described. Inhibition of Gram-positive and Gram-negative bacteria including β-lactamase producing strains was observed with phenyl as the aryl residue. The 3,4-dihydroxyphenyl group further enhanced the activity against Gram-negative organisms; in this series, the 3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl] and 3-[(1-carboxymethyl-1*H*-tetrazol-5-yl)thiomethyl] analogues (**2** and **12b**) exhibited exceptional activity against Gram-negative bacteria, including *Pseudomonas aeruginosa*.

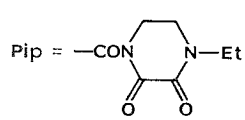
Recent reports from these laboratories describe the synthesis[1] and antibacterial activity[2] of several 7α-formamidocephalosporins. The 7α-formamido moiety, in contrast to the 7α-methoxyl group in cephamycins[3] generally confers excellent β-lactamase stability without compromising the antimicrobial activity of semi-synthetic derivatives. In particular, the 7β-acylamino-7α-formamidocephalosporin derivatives (**1** and **2**) (Fig. 1)[1,4] are broad-spectrum, β-lactamase stable antimicrobial agents highly active against Gram-negative bacteria, including *Pseudomonas aeruginosa*.[2] This finding prompted the preparation of further 3-(heterocyclylthio)methyl and 3-(pyridinium)methyl analogues. This paper describes our initial studies in this area.

Fig. 1.



1 R = H

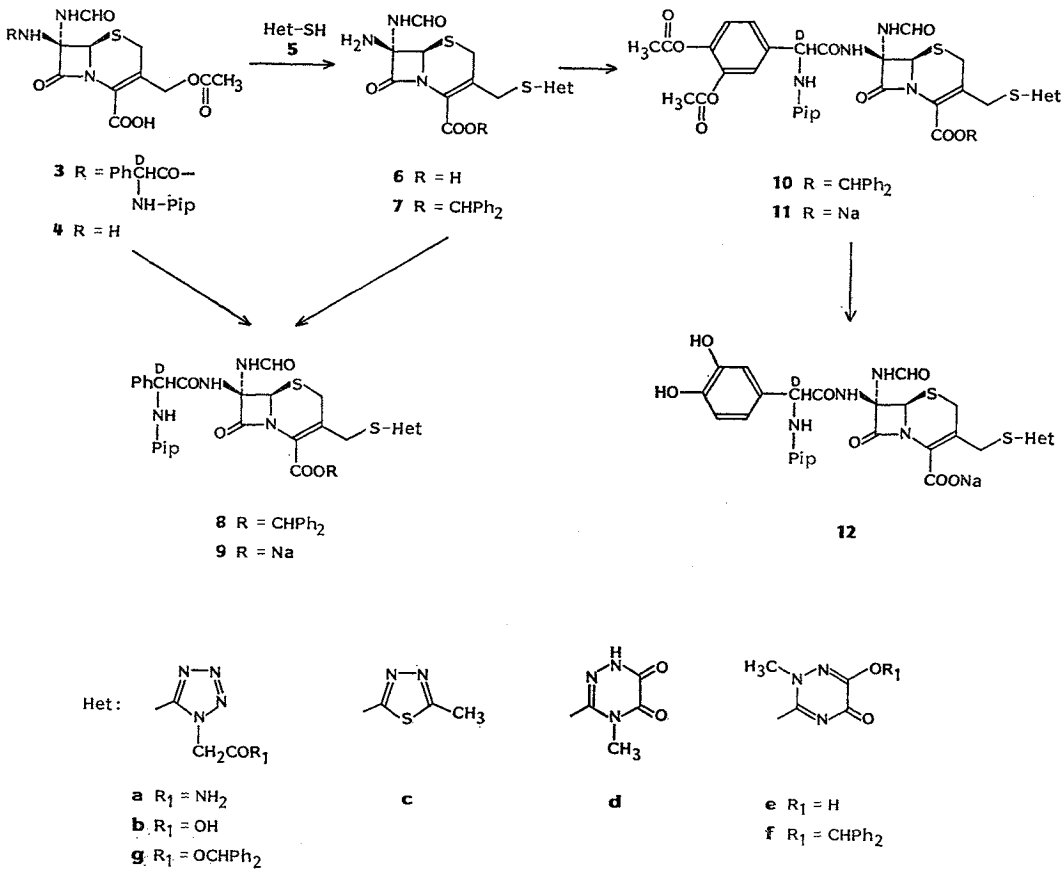
2 R = OH



Chemistry

The preparation of the required 7β-acylamino derivatives was similar to that previously described,[1] and is outlined in the Scheme 1. Although the 7β-acylamino-7α-formamidocephalosporanic acid (**3**)[1] could be converted directly into the 3-(1-carbamoylmethyl-1*H*-tetrazolyl)-thiomethyl analogue (**9a**) *via* acetate displacement with the thiol (**5a**), the process was poor, and not generally applicable. A more versatile procedure utilised the 7β-amino-7α-formamidocephalosporanic acid (**4**). Thus, the C-3 heterocyclyl-

Scheme 1.



thio functionality (**b**~**d**) was readily incorporated into **4** by reaction with the appropriate thiols (**5b**~**5d**) in acidic aqueous acetone. The acids (**6b**~**6d**), purified *via* their sodium salts on Diaion HP-20 SS resin, were then converted to the corresponding benzhydryl esters (**7g**, **7c** and **7d**). In contrast, introduction of the 2-methyl-1,2,4-triazinylthio moiety (**e**) proved less straightforward. The standard displacement-esterification sequence using **5e** gave none of the expected ester (**7e**), but a low yield of the ester-ether (**7f**). The concomitant etherification reflects the acidic nature of the triazinyl hydroxyl group in **6e** relative to the isomer (**6d**). It was surmised that initial hydroxyl protection might prove advantageous and accordingly (**5e**) was selectively *O*-etherified to the thiol (**5f**). Subsequent reaction with the acid (**4**), although poor under acidic conditions, proceeded at neutral pH to give the required ester-ether (**7f**) in good yield following esterification. ALPEGIANI *et al.*[5] have recently reported similar observations on the relative acidities of triazinones (**5d** and **5e**), and the necessity for *O*-silyl protection of the latter in penem synthesis.

The amines (**7g**, **7c** and **7d**) were then acylated with D-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetyl chloride,[1] and the

Fig. 2.

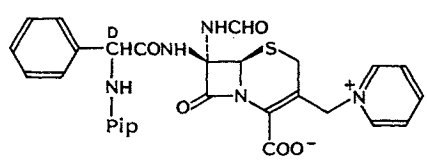


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Table 1. Antibacterial activity (MIC μg/ml) of 7α-formamidocephalosporins.

Organism	**1**	**9a**	**9b**	**9c**	**9d**	**15**	**2**	**12b**	**12c**	**12e**	CPZ	CAZ
Escherichia coli NCTC 10418	0.06	0.06	0.06	0.06	0.12	0.06	≤0.03	≤0.03	≤0.03	≤0.03	0.06	0.06
E. coli DCO RTEM[a]	0.12	0.12	0.12	0.12	0.5	1	≤0.03	≤0.03	≤0.03	≤0.03	1	0.25
E. coli JT425[b]	0.5	0.12	0.25	0.5	2	4	≤0.03	≤0.03	≤0.03	≤0.03	2	8
Enterobacter cloacae N1	1	1	0.25	2	2	4	0.25	0.12	2	2	2	0.25
E. cloacae P99[b]	—	—	—	—	4	8	2	8	2	4	128	128
Klebsiella pneumoniae T767[b]	0.5	0.5	0.25	0.5	1	1	≤0.03	≤0.03	≤0.03	≤0.03	0.25	0.25
Proteus mirabilis C977	0.5	0.5	0.25	1	2	4	0.12	0.12	4	2	0.25	0.12
Serratia marcescens US32	0.25	0.12	0.5	1	1	2	0.12	0.25	1	0.5	2	0.5
S. marcescens HCN 3956	8	4	2	16	8	16	1	4	4	2	>128	8
Pseudomonas aeruginosa NCTC 10662	8	8	4	8	16	16	0.25	0.12	1	0.5	4	1
P. aeruginosa Dalgleish[a]	8	8	4	8	8	8	0.25	0.12	1	1	32	1
P. aeruginosa Badia	—	—	—	—	16	8	0.25	0.25	1	1	>128	64
Staphylococcus aureus Oxford	8	8	64	8	4	8	16	64	32	>32	2	8
S. aureus Russell[a]	8	8	64	8	8	8	16	>64	32	>32	4	16
Streptococcus pyogenes CN10	0.12	0.12	4	0.06	0.06	0.06	2	8	2	16	0.12	0.12

[a] Plasmid-mediated β-lactamase-producing strain.
[b] Non-plasmid-mediated β-lactamase-producing strain.

resulting amides (**8g**, **8c** and **8d**) deprotected to afford the corresponding sodium salts (**9b**~**9d**) respectively. Similarly, the amines (**7g**, **7c** and **7f**) were progressed *via* the amides (**10g**, **10c** and **10f**) to the sodium salts (**11b**, **11c** and **11e**). Final deprotection with sodium sulfite[6] provided the catecholic derivatives (**12b**, **12c** and **12e**).

The preparation of the 3-(pyridinium)methyl- analogue (**15**) (Fig. 2) has been reported.[1]

Results and Discussion

The minimum inhibitory concentrations (MICs) of the 3-(pyridinium)methyl- and the various 3-(heterocyclylthio)methylcephalosporin derivatives against a range of Gram-positive and Gram-negative bacteria, including ceftazidime-resistant strains, were determined using an agar dilution method and are shown in Table 1. Cefoperazone (CPZ) and ceftazidime (CAZ) were included as reference compounds.

In the unsubstituted aryl series, the *N*-carbamoylmethyltetrazolyl- and the 2-methylthiadiazolyl compounds (**9a** and **9c**) had similar broad-spectrum activity to the lead compound (**1**), but the tetrazole derivative (**9a**) was more potent against *Escherichia coli* JT425. In addition, against *Serratia marcescens*, **9a** was respectively 2-fold and 4~8-fold more active than **1** and **9c**. The *N*-carboxymethyltetrazolyl substituent of **9b** slightly improved the overall potency against Gram-negative organisms including *P. aeruginosa*, but reduced activity against Gram-positive bacteria. In contrast, the triazinyl and pyridinium analogues (**9d** and **15**), whilst comparable to **1** against Gram-positive cocci, were 2~4-fold less potent against members of the family Enterobacteriaceae and *P. aeruginosa*.

The enhanced activity against Gram-negative bacteria produced by 3,4-dihydroxy substitution in the side-chain phenyl residue can be seen by comparison of the activities of **1** and **2**. A similar effect is clearly evident for **12b** and **12c** compared to **9b** and **9c**, respectively. These catecholic analogues, and also **12e**, were exceptionally potent (MIC $\leq$0.03 μg/ml) against *E. coli*, including chromosomally- and plasmid-mediated β-lactamase producing strains and *Klebsiella pneumoniae*. Against other bacteria, the level of antimicrobial activity was dependent on the nature of the C-3 substituent. The *N*-carboxymethyltetrazolyl compound (**12b**) was more active than **2** against *P. aeruginosa*, but some reduction in activity against *Enterobacter cloacae* P99 and *S. marcescens* HCN 3956 was evident. In contrast, the thiadiazolyl and triazinyl analogues (**12c** and **12e**) were less potent overall than the tetrazole (**2**), although still very active against *P. aeruginosa*, including *P. aeruginosa* Badia, a ceftazidime-resistant strain.

In summary, all the 7α-formamidocephalosporins described showed an excellent combination of antibacterial activity and β-lactamase stability, and were similar to or more active than the standard compounds against Gram-negative organisms. The antibacterial activity against *Staphylococcus aureus* was inferior to cefoperazone, but comparable, in many cases, to ceftazidime. The phenyl derivatives demonstrated moderate broad-spectrum activity whereas the dihydroxyphenyl compounds possessed excellent activity against Gram-negative organisms including *P. aeruginosa*. Overall, the tetrazoles (**2** and **12b**) were the most potent agents against Gram-negative bacteria and were significantly more active than ceftazidime against strains of *P. aeruginosa*.

Experimental

IR spectra were recorded for dichloromethane solutions on a Perkin-Elmer 197 spectrophotometer and for KBr discs on Perkin-Elmer 457 or Perkin-Elmer 983 grating spectrophotometers. ^{1}H NMR spectra were obtained on Perkin-Elmer R32 (90 MHz) or Brucker WM 250 (250 MHz) instruments

Table 2. ^{1}H NMR and IR spectral data.

Compound No.	^{1}H NMR (250 MHz) (Solvent) δ (J; Hz)	IR $\nu_{C=O}^{KBr}$ (cm^{-1}) (β-lactam)
8c	($(CD_3)_2CO$); 1.17 (3H, t, J=7), 2.62 (3H, s), 3.16 and 3.29 (2H, ABq, J=16), 3.49 (2H, q, J=7), 3.70 (2H, m), 4.05 (2H, m), 4.29 and 4.65 (2H, ABq, J=14), 5.29 (1H, s), 5.73 (1H, d, J=7, collapses to s on exchange), 6.90 (1H, s), 7.20~7.70 (15H, m), 8.29 (1H, d, J=1, collapses to s on exchange), 8.53 (1H, br s, exchange), 8.74 (1H, s, exchange), 10.02 (1H, d, J=7, exchange)	1785[a]
9c	(D_2O); 1.18 (3H, t, J=7), 2.72 (3H, s), 2.99 and 3.40 (2H, ABq, J=17), 3.47 (2H, q, J=7), 3.69 (2H, m), 3.90 and 4.36 (2H, ABq, J=14), 4.00 (2H, m), 5.23 (1H, s), 5.51 (1H, s), 7.30~7.60 (5H, m), 8.12 (1H, s)	1770
10f	($(CD_3)_2CO$); 1.16 (3H, t, J=7), 2.09 (3H, s), 2.18 (3H, s), 3.01 and 3.29 (2H, ABq, J=16), 3.49 (2H, q, J=7), 3.60 (3H, s), 3.65 (2H, m), 4.05 (2H, m), 4.16 and 4.59 (2H, ABq, J=13), 5.26 (1H, s), 5.74 (1H, d, J=7, collapses to s on exchange), 6.87 (1H, s), 6.96 (1H, s), 7.20~7.70 (23H, m), 8.30 (1H, d, J=1, collapses to s on exchange), 8.49 (1H, br s, exchange), 8.88 (1H, s, exchange), 10.08 (1H, d, J=7, exchange)	1780[a]
11e	(D_2O); 1.19 (3H, t, J=7), 2.25 (3H, s), 2.29 (3H, s), 3.50 (2H, q, J=7), 3.72 (3H, s), 3.5~4.1 (6H, m), 4.53 (2H, AA'), 5.38 (1H, s), 5.53 (1H, s), 7.20~7.50 (3H, m), 8.15 (1H, s)	1770
12c	(D_2O); 1.16 (3H, t, J=7), 2.68 (3H, s), 3.01 and 3.39 (2H, ABq, J=17), 3.47 (2H, q, J=7), 3.70 (2H, m), 3.78~4.10 (3H, m), 4.28 (1H, d, J=14), 5.23 (1H, s), 5.31 (1H, s), 6.80~7.05 (3H, m), 8.10 (1H, s)	1770
12e	(D_2O); 1.19 (3H, t, J=7), 3.03 and 3.39 (2H, ABq, J=17), 3.51 (2H, q, J=7), 3.62 (3H, s), 3.68 (2H, m), 4.02 (3H, m), 4.29 (1H, d, J=14), 5.27 (1H, s), 5.33 (1H, s), 6.85~7.05 (3H, m), 8.13 (1H, s)	1765

[a] IR (CH_2Cl_2).

using TMS as internal standard, except for D_2O solutions when HOD (250 MHz) was used as internal standard. While two rotameric forms were observed in the ^{1}H NMR spectra, only the major, *Z*, rotamer is quoted. Mass spectra were recorded on either a VG 7070 or a VG ZAB spectrometer operating in the electron impact mode. Fast atom bombardment spectra were recorded on a VG ZAB spectrometer and the matrix used is stated. Preparative chromatography was carried out on Silica gel 60 (finer than 230 mesh ASTM) (Merck 7729). Solvents were dried prior to use and evaporated under reduced pressure below 30°C.

In Vitro Antibacterial Activity

MICs were determined by serial dilution in Iso-sensitest agar containing 5% defibrinated horse blood, inoculated with about 10^4 cfu for Gram-negative bacteria and about 10^6 cfu for Gram-positive bacteria, and incubated overnight at 37°C.

Materials

The preparation of compounds **7d**, **7g**, **8d**, **8g**, **9a**, **9b**, **9d**, **10g**, **11b**, **12b**,[6] **7c**, **10c** and **11c**[7] has been detailed in the patent literature. The general synthetic procedures described therein were utilised to prepare the new analogues **8c**, **9c**, **10f**, **11e**, **12c** and **12e**: ^{1}H NMR and IR spectral data of which are listed in Table 2.

2,5-Dihydro-6-diphenylmethoxy-3-mercapto-2-methyl-5-oxo-1,2,4-triazine (**5f**)

Diphenyldiazomethane (0.7 g, 3.6 mmol) was added portionwise with stirring to a suspension of **5e** (0.6 g, 3.8 mmol) in acetonitrile (10 ml). After a further 10 minutes, the resulting solution was

evaporated and the residual gum chromatographed to afford **5f** (1.0 g, 82%): UV λ_{max}^{EtOH} nm (ε) 272 (20,974); IR (KBr) cm^{-1} 3415, 1720, 1600; ^1H NMR (90 MHz, $(CD_3)_2CO$) δ 3.65 (3H, s), 6.82 (1H, s), 7.20~7.60 (10H, m); MS *m/z* 325 (M, $C_{17}H_{15}N_3O_2S$).

Diphenylmethyl 7β-Amino-3-[(2,5-dihydro-6-diphenylmethoxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)-thiomethyl]-7α-formamidoceph-3-em-4-carboxylate (**7f**)

To a solution of 7β-amino-7α-formamidocephalosporanic acid (**4**) (0.371 g, 1.1 mmol) in water (10 ml) at pH 7.0 was added **5f** (0.282 g, 0.9 mmol) in THF (10 ml) and the mixture heated to 60°C under argon for 6 hours. The reaction mixture was filtered, the filtrate concentrated and the aqueous solution acidified to pH 1.1 with 5 N hydrochloric acid. The precipitated crude acid (**6f**) was filtered off, suspended in acetonitrile (20 ml), and treated with diphenyldiazomethane (0.9 g, 4.6 mmol) for 16 hours. Acetic acid was added to neutralise residual diphenyldiazomethane, the solution evaporated and the residue partitioned between dichloromethane (50 ml) and saturated aqueous sodium bicarbonate (10 ml). The organic phase was separated, dried ($MgSO_4$), evaporated and the crude product chromatographed to afford (**7f**) (0.266 g, 41%): IR (CH_2Cl_2) cm^{-1} 3400, 1780, 1720, 1690, 1670; ^1H NMR (250 MHz, $CDCl_3$) δ 1.57 (2H, br s, exchange), 3.46 and 3.63 (2H, ABq, J=17 Hz), 3.54 (3H, s), 4.15 and 4.48 (2H, ABq, J=13 Hz), 5.16 (1H, s), 6.35 (1H, br s, exchange), 6.75 (1H, s), 6.97 (1H, s), 7.2~7.5 (20H, m), 8.26 (1H, d, J=0.7 Hz, collapses to s on exchange); fast atom bombardment mass spectrum (FAB-MS) (positive xenon; thioglycerol) *m/z* 747 (M+H, $C_{39}H_{34}N_6O_6S_2$).

The ester (**7f**) was then progressed *via* **10f** and **11f** to **12e** using the procedures previously described.[6,7]

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