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Research Papers

Synthesis and antibacterial activity of fluorobenzoylcephalosporins

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Summary

The atoms of halogens are very frequently present at C(3) or C(3′) in forms such as 3-halo-, 3-halomethyl- or 3-halovinylcephems, which are especially useful for nucleophilic substitution, but compounds with one or more halogen atom at C(7β) are rare. A group of fluorobenzoylcephalosporins have been prepared from 7-ACA and 7-ADCA, and a group of compounds, where the 3′-acetoxy group was substituted by an S-nucleophile, i.e. imidazole, 1,2,4-triazole or 1,3,4-thiadiazole. The dilution method (the minimal inhibitory concentration) was used to test the intermediates (products of acylation) and the final product of S-nucleophilic substitution. The results indicate that nucleophilic substitution increases the in vitro antibacterial activity of 2,6-$(F)_2$- and 2,3,4,5,6-$(F)_5$- derivatives, but decreases the activity of 2-F derivatives.

Introduction

The chemical nature of the 7β-acylamido side chain of cephalosporins and its spatial orientation are of great importance for antibacterial activity. Cephalosporins of different generations possess typical 7-acylamido side chains, which determine the antibacterial profile by creating characteristic affinities to the PBPs and by influencing the permeation properties across the outer membrane of bacteria and β-lactamase stability (Dürckheimer et al., 1988).

In the field of cephalosporin, flomoxef (**1**) and 2355-S (**2**) are known antibiotics in which one or two atoms of fluorine are present at the 7β-position. These compounds, oxacephems, have an in vitro antibacterial spectrum which was elucidated in comparison with cefotaxime, latamoxef and cefoperazone and other agents (Dürckheimer et al., 1988). The most remarkable feature of flomoxef is its high antistaphylococcal activity, including several clinical isolates of methicillin-resistant staphylococci.

We previously reported the synthesis and antibacterial activity of fluorobenzoyldeacetoxy-

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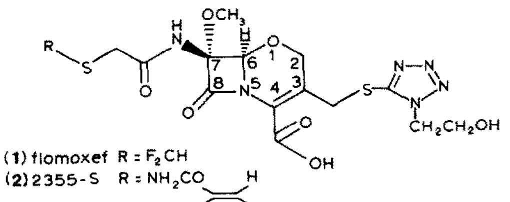
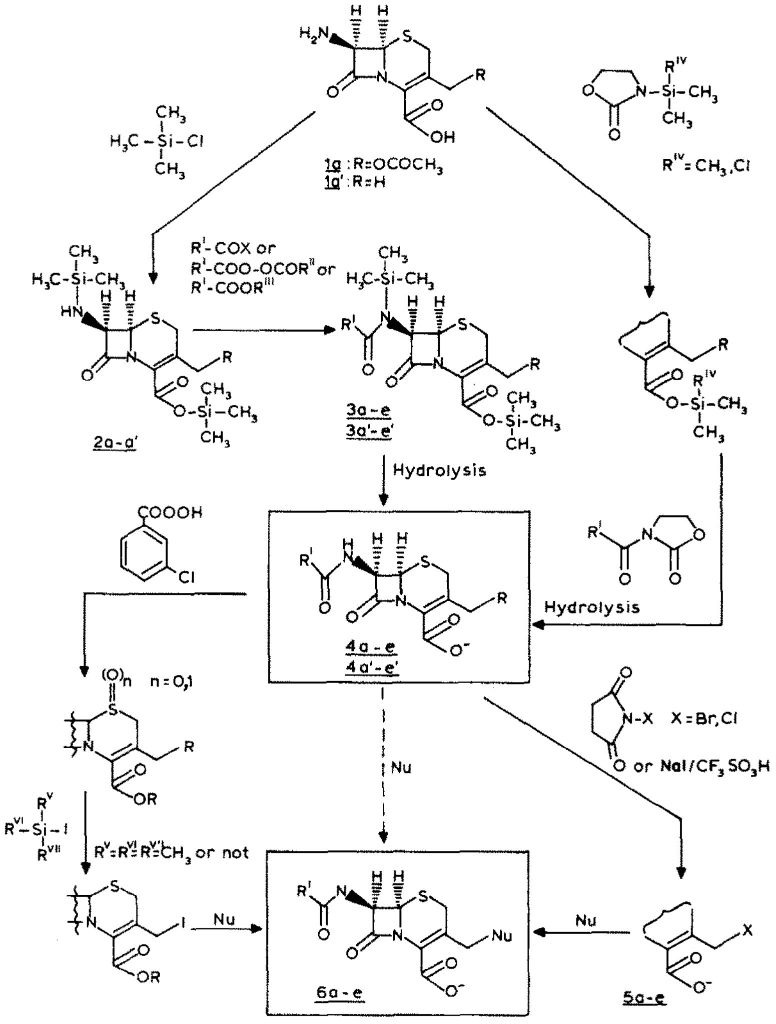


Fig. 1. Flomoxef and 2355-S, and numbering of cephem nucleus.

cephalosporins. A survey of the syntheses of these compounds established that a relatively small number of fluorobenzoylcephalosporins are known which have one or more fluorine atom at the 7β-position. General synthetic routes are shown in Scheme 1. This scheme was developed by using different synthetic methods which have been described in patent literature (Kennedy et



Scheme 1. General synthetic routes for preparation of fluorobenzoylcephalosporins and fluorobenzoyldeacetoxycephalosporins.

al., 1970; Long et al., 1970; Pechmann et al., 1976; Vitezić et al., 1979; Wright, 1979; Nagano et al., 1984) and in our papers (Kobal and Valenčič, 1988; Kobal et al., 1988, 1989a,b, 1990; Kobal, 1991).

The plan of investigations in the field of synthetic works

We used the most common route for semisynthetic cephalosporins starting from 7-ACA or 7-ADCA as follows:

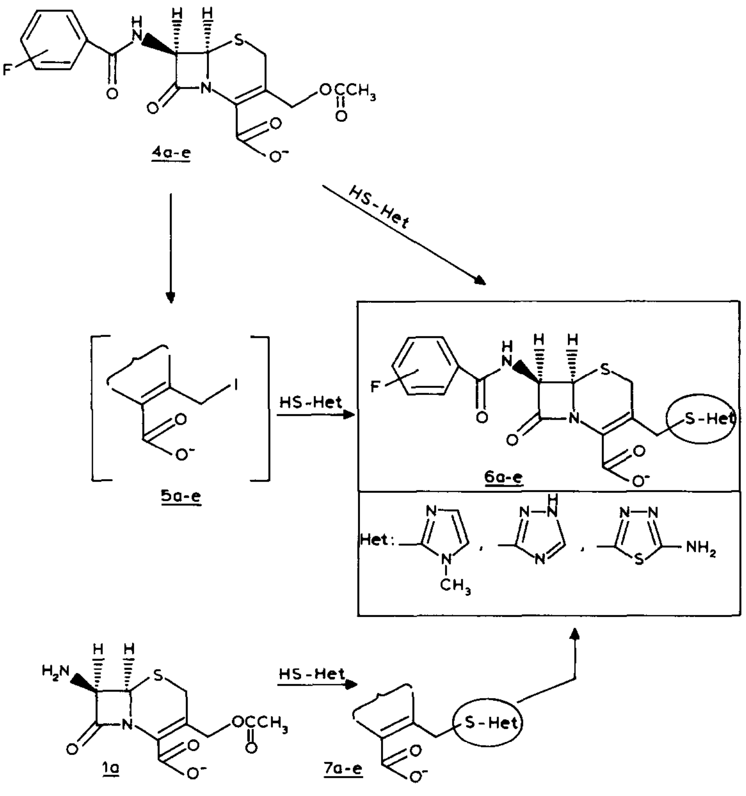
synthesis of the 7-side chain acid and, if necessary (in some variations of the general method for acylation by acid chlorides), protection of functional groups;

coupling of the side chain acid to the 7-aminocephem nucleus optionally followed by deprotection of functional groups;

displacement of the 3′-acetoxy residue in 7-ACA by S-nucleophiles; preparation of stable sodium salts (Kobal et al., 1988; Valenčič, 1988).

The acylation of 7-ACA or 7-ADCA with different acid chlorides yielded the desired intermediates with a halosubstituted phenyl ring at the 7-aminoacyl side chain, which we used for nucleophilic substitution via direct and indirect methods. Displacement of the 3′-acetoxy group by S-nucleophiles is usually achieved in aqueous solution (pH about 6.0) between 40 and 90 °C (Dürckheimer et al., 1988). General routes for the preparation of 3-thiomethylcephalosporins are outlined in Scheme 2.

We used aqueous solutions and temperatures between 65 and 67 °C. In the preparation of 3-iodomethyl intermediates with fluorobenzoyl-cephalosporin reaction intermediates (products of the first step of the reaction) with sodium iodide



Scheme 2. Synthesis of 3-thiomethylcephalosporins ($6a_1$–a_3, $6d_1$–d_3, $6e_1$–e_3) on different routes, described in the text.

and trifluoromethanesulfonic acid in aprotic solvents, e.g. acetonitrile, displacement of the 3′-acetoxy group by S-nucleophiles was achieved in aqueous solution (pH about 6.0) at 45 ° C (Kobal, 1991).

The plan of investigations in the field of biological evaluation of synthesized compounds

We were interested in the following questions:

(1) Is this type of substance (a fluorobenzoylcephalosporin) highly active only against Gram-positive organisms?
(2) Is S-nucleophilic substitution at C(3′) (displacement of the 3′-acetoxy group with S-nucleophiles) advantageous or is activity lower than for the corresponding unsubstituted compounds at C(3′)?
(3) Which S-nucleophile: imidazole, 1,2,4-triazole or 1,3,4-thiadiazole is especially favorable?

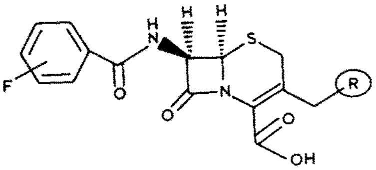
Materials and Methods

Chemistry: general

Melting points were determined with a Kofler microscope and are uncorrected. ^{1}H-NMR spectra were obtained at 60 MHz using a Varian EM

TABLE 1

*Analytical, m.p. and IR data of compounds (**4a–e, 4a′–e′**)*



Compound no.	F	R	Yield (%)	Analysis Calcd.: Found:	m.p. (dec., ° C)	IR (KBr) $\nu_{\max} C = 0$ (β-lactam) (cm^{-1})
4a	2-F	$OCOCH_3$	71	C 51.66, H 3.83, N 7.09 C 52.11, H 3.83, N 7.01	152–155	1790
4a′	2-F	H	66	C 53.57, H 3.89, N 8.33 C 53.63, H 4.04, N 8.20	177–180	1785
4b	3-F	$OCOCH_3$	63	C 51.66, H 3.83, N 7.09 C 51.20, H 3.65, N 7.00	110–112	1795
4b′	3-F	H	60	C 53.57, H 3.89, N 8.33 C 53.50, H 3.90, N 8.05	150–154	1790
4c	4-F	$OCOCH_3$	45	C 51.66, H 3.83, N 7.09 C 50.95, H 3.80, N 6.85	148	1795
4c′	4-F	H	41	C 53.57, H 3.89, N 8.33 C 53.28, H 3.55, N 8.25	132–135	1795
4d	2,6-$(F)_2$	$OCOCH_3$	50	C 49.51, H 3.42, N 6.79 C 49.38, H 3.20, N 6.80	148–150	1795
4d′	2,6-$(F)_2$	H	78	C 50.84, H 3.41, N 7.90 C 50.55, H 3.25, N 7.45	175–178	1790
4e	2,3,4,5,6-$(F)_5$	$OCOCH_3$	53	C 43.78, H 2.38, N 6.00 C 43.70, H 2.50, N 5.85	120–124	1790
4e′	2,3,4,5,6-$(F)_5$	H	62	C 44.12, H 2.22, N 6.86 C 44.08, H 2.17, N 6.45	128–131	1785

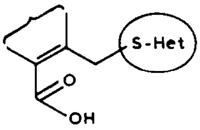
TABLE 2

*^{1}H-NMR data of compounds (**4a–e, 4a′–e′**)*

Compound no.	^{1}H-NMR (DMSO-d$_6$) (ppm)
4a	2.00 (3H,s,OCOCH$_3$), 3.45 (2H,s,CH$_2$), 4.90 (1H,d,C(6)-H), 5.45 (1H,dd,C(7)-H), 7.25 (4H,s,C$_6$H$_4$F), 9.25 (1H,d,NH-CO)
4a′	1.80 (3H,s,CH$_3$), 3.40 (2H,s,CH$_2$), 4.90 (1H,d,C(6)-H), 5.45 (1H,dd,C(7)-H), 7.50 (4H,s,C$_6$H$_4$F), 8.95 (1H,d,NH-CO)
4b	2.10 (3H,s,OCOCH$_3$), 3.55 (2H,s,CH$_2$), 5.20 (1H,d,C(6)-H), 5.85 (1H,dd,C(7)-H), 7.50 (4H,m,C$_6$H$_4$F), 9.45 (1H,d,NH-CO)
4b′	1.80 (3H,s,CH$_3$), 3.40 (2H,s,CH$_2$), 5.00 (1H,d,C(6)-H), 5.45 (1H,dd,C(7)-H), 7.45 (4H,m,C$_6$H$_4$F), 9.05 (1H,d,NH-CO)
4c	2.10 (3H,s,OCOCH$_3$), 3.55 (2H,s,CH$_2$), 5.20 (1H,d,C(6)-H), 5.85 (1H,dd,C(7)-H), 7.65 (4H,s,C$_6$H$_4$F), 9.40 (1H,d,NH-CO)
4c′	1.85 (3H,s,CH$_3$), 3.45 (2H,s,CH$_2$), 4.95 (1H,d,C(6)-H), 5.50 (1H,dd,C(7)-H), 7.25 (4H,s,C$_6$H$_4$F), 9.20 (1H,d,NH-CO)
4d	1.90 (3H,s,OCOCH$_3$), 3.50 (2H,s,CH$_2$), 4.95 (1H,d,C(6)-H), 5.65 (1H,dd,C(7)-H), 7.30 (3H,m,C$_6$H$_3$F$_2$), 9.45 (1H,d,NH-CO)
4d′	2.00 (3H,s,CH$_3$), 4.30 (2H,s,CH$_2$), 5.10 (1H,d,C(6)-H), 5.70 (1H,dd,C(7)-H), 7.35 (3H,m,C$_6$H$_3$F$_2$), 9.80 (1H,d,NH-CO)
4e	2.00 (3H,s,OCOCH$_3$), 3.90 (2H,s,CH$_2$), 5.05 (1H,d,C(6)-H), 5.60 (1H,dd,C(7)-H), 9.25 (1H,d,NH-CO)
4e′	2.00 (3H,s,CH$_3$), 4.15 (2H,s,CH$_2$), 4.90 (1H,d,C(6)-H), 5.75 (1H,dd,C(7)-H), 9.15 (1H,d,NH-CO)

TABLE 3

*Analytical, m.p. and IR data of compounds (**6a$_1$–a$_3$, 6d$_1$–d$_3$, 6e$_1$–e$_3$**)*



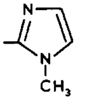
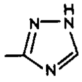
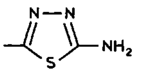
Compound no.	F	Het	Yield (%)	Analysis Calcd.: Found:	m.p. (dec., °C)	IR (KBr) $\nu_{max}c = 0$ (β-lactam) (cm^{-1})
6a$_1$	2-F		35	C 50.88, H 3.82, N 12.49 C 50.20, H 3.80, N 11.98	110–112	1790
6d$_1$	2,6-(F)$_2$		45	C 48.92, H 3.46, N 12.01 C 48.56, H 3.35, N 11.85	192–195	1790
6e$_1$	2,3,4,5,6-(F)$_5$		34	C 43.85, H 2.52, N 10.76 C 43.10, H 2.43, N 10.81	> 205	1770
6a$_2$	2-F		33	C 46.99, H 3.02, N 16.12 C 46.23, H 2.88, N 15.80	178–182	1800
6d$_2$	2,6-(F)$_2$		16	C 45.13, H 2.67, N 15.48 C 44.76, H 2.59, N 15.02	182–185	1785
6e$_2$	2,3,4,5,6-(F)$_5$		20	C 40.32, H 1.79, N 13.83 C 40.02, H 1.65, N 13.62	179–182	1785
6a$_3$	2-F		72	C 43.67, H 3.02, N 14.98 C 43.24, H 2.98, N 14.67	189–194	1800
6d$_3$	2,6-(F)$_2$		53	C 42.05, H 2.70, N 14.42 C 41.97, H 2.43, N 14.13	138–142	1795
6e$_3$	2,3,4,5,6-(F)$_5$		13	C 37.85, H 1.87, N 12.98 C 37.43, H 1.67, N 12.66	128–132	1775

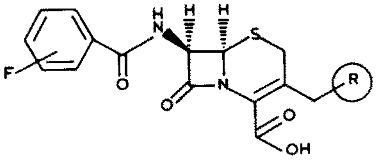
TABLE 4

*^{1}H-NMR data of compounds (**$6a_1$–a_3, $6d_1$–d_3, $6e_1$–e_3**)*

Compound no.	^{1}H-NMR ($DMSO-d_6$) (ppm)
$6a_1$	3.30 (3H,s,imidazole-N-CH_3), 3.45 (2H,s,CH_2), 4.00 (2H,s,CH_2), 5.20 (1H,d,C(6)-H), 5.85 (1H,dd,C(7)-H), 6.80 (1H,d,imidazole-H), 7.00 (1H,d,imidazole-H), 7.50 (4H,s,C_6H_4F), 9.75 (1H,d,NH-CO)
$6d_1$	3.05 (3H,s,imidazole-N-CH_3), 3.40 (2H,s,CH_2), 4.00 (2H,s,CH_2), 5.20 (1H,d,C(6)-H), 5.70 (1H,dd,C(7)-H), 7.00 (1H,d,imidazole-H), 7.20 (1H,d,imidazole-H), 8.00 (3H,m,$C_6H_3F_2$), 9.50 (1H,d,NH-CO)
$6e_1$	3.25 (3H,s,imidazole-N-CH_3), 3.65 (2H,s,CH_2), 4.00 (2H,s,CH_2), 5.10 (1H,d,C(6)-H), 5.70 (1H,dd,C(7)-H), 6.80 (1H,d,imidazole-H), 7.20 (1H,d,imidazole-H), 9.15 (1H,d,NH-CO)
$6a_2$	3.80 (2H,s,CH_2), 4.20 (2H,s,CH_2), 5.05 (1H,d,c(6)-H), 5.70 (1H,dd,C(7)-H), 7.45 (4H,s,C_6H_4F), 8.30 (1H,d,triazole-H), 8.50 (1H,d,triazole-H), 9.60 (1H,d,NH-CO)
$6d_2$	3.80 (2H,s,CH_2), 4.20 (2H,s,CH_2), 5.15 (1H,d,C(6)-H), 5.85 (1H,dd,C(7)-H), 7.20 (3H,m,$C_6H_3F_2$), 8.30 (1H,d,triazole-H), 8.45 (1H,d,triazole-H), 9.80 (1H,d,NH-CO)
$6e_2$	3.60 (2H,s,CH_2), 4.60 (2H,s,CH_2), 5.35 (1H,d,C(6)-H), 6.00 (1H,dd,C(7)-H), 7.95 (1H,d,triazole-H), 8.10 (1H,d,triazole-H), 9.80 (1H,d,NH-CO)
$6a_3$	3.80 (2H,s,CH_2), 4.25 (2H,s,CH_2), 5.15 (1H,d,C(6)-H), 5.80 (1H,dd,C(7)-H), 7.40 (4H,s,C_6H_4F), 9.40 (1H,d,NH-CO)
$6d_3$	3.80 (2H,s,CH_2), 4.20 (2H,s,CH_2), 5.15 (1H,d,C(6)-H), 5.50 (1H,dd,C(7)-H), 7.40 (3H,m,$C_6H_3F_2$), 9.70 (1H,d,NH-CO)
$6e_3$	3.60 (2H,s,CH_2), 4.00 (2H,s,CH_2), 5.30 (1H,d,C(6)-H), 5.70 (1H,dd,C(7)-H), 9.65 (1H,d,NH-CO)

TABLE 5

*Antibacterial activity (MIC, $\mu g\ ml^{-1}$) of compounds — 'intermediates' (**4a–a′, 4d–d′, 4e–e′**)*



Compound no.	**4a**	**4a′**	**4d**	**4d′**	**4e**	**4e′**	Cephalothin	Cefuroxime
R	$OCOCH_3$	H	$OCOCH_3$	H	$OCOCH_3$	H		
F	2-F	2-F	2,6-$(F)_2$	2,6-$(F)_2$	2,3,4,5,6-$(F)_5$	2,3,4,5,6-$(F)_5$		
S. aureus	$\boxed{31.2}$	–	–	–	–	150	500	–
S. epidermidis	$\boxed{62.5}$	–	–	–	–	500	500	–
Streptococcus G	125	–	–	500	500	–	500	500
Strep. agalactiae	250	–	500	–	500	500	125	125
E. coli T18	$\boxed{62.5}$	500	500	500	500	500	62.5	500
P. mirabilis	125	–	500	–	–	250	125	62.5
K. pneumoniae	250	–	500	–	–	–	500	500
Ps. aeruginosa	125	–	–	500	500	250	500	500
H. influenzae	$\boxed{62.5}$	–	125	–	–	125	250	250

360 spectrometer. IR spectra were taken on a Perkin Elmer 257 spectrophotometer. In addition, a Perkin Elmer 240C CHN Analyzer was applied to the elemental analyses of C, H and N.

*General procedure for preparation of fluorobenzoylcephalosporins (**4a–e, 4a′-e′**) (Scheme 1)*

7-Amino-3-acetoxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**1a**, 0.040 mol) was suspended in dichloromethane (200 ml) and bis[(trimethyl)silyl]acetamide (14 ml, 0.057 mol) was added dropwise. A solution of fluorobenzoylchloride (0.040 mol) was then added dropwise. Consequently, the mixture was stirred at room temperature for 2 h.

This stage was followed by adding water (150 ml) to the prepared solution which was in turn stirred again, this time for 30 min.

The desired pH of the aqueous phase, which in this instance was 6.5–7.0, was achieved by gradual addition of potassium bicarbonate.

The organic phase was separated and washed with water (50 ml). The combined aqueous phases were acidified to pH 2.0 by 2 N HCl. The precipitates were filtered, washed with water and dried in vacuo.

The analytical, m.p. and IR data of compounds (**4a–e, 4a′–e′**) are reported in Table 1; ^{1}H-NMR data are listed in Table 2.

General procedure for S-nucleophilic substitution of acylated products ($6a_1$–e_1, $6a_2$–e_2, $6a_3$–e_3) (Scheme 2)

Method A

First step: preparation of 3-iodomethyl derivatives Fluorobenzoylcephalosporin intermediate (0.0025 mol), sodium iodide (0.0040 mol) and acetonitrile (4 ml) were placed in a 10 ml flask and the mixture was cooled until the inside temperature reached 18 °C. Then trifluoromethanesulfonic acid (1 ml) was added dropwise to the mixture at the same temperature as above over a 10 min period. After conducting a reaction for 20 min at room temperature, the reaction mixture was dispersed in ice water (20 ml). The dispersion was stirred for 10 min under ice-cooling and then the precipitates were recovered by filtration, washed with water (5 ml) and dried over phosphorus pentoxide in a desiccator.

Second step: S-nucleophilic substitution of 3-iodomethyl derivatives 3-Iodomethyl derivative (0.0004 mol) was suspended in water (5 ml) and sodium hydrogen carbonate (0.0004 mol) was added to the suspension. To the solution was added S-nucleophile (0.0012 mol) and the mixture was allowed to react for 180 min at 45 °C. After the reaction had reached completion, the mixture was adjusted to pH 2.5 with 2 N HCl under ice-cooling. After stirring the reaction mixture for 10 min under ice-cooling, the precipitates thus formed were recovered by filtration. The precipitates were washed with cold water (10 ml) and dried under vacuum.

Method B

Compounds (**6a–e**) were obtained in a manner similar to that described above by direct S-nucleophilic substitution of fluorobenzoylcephalosporin intermediates with S-nucleophiles (only the second step of method A).

The analytical, m.p. and IR data of compounds (**6a–e, 6a′–e′**) are reported in Table 3 and the ^{1}H-NMR data are given in Table 4.

Biological screening

Serial dilutions of synthesized compounds are inoculated with the organisms and incubated. For routine dilution procedures, it is generally unnecessary and inadvisable to test more than one representative from a group of related organisms (Washington and Barry, 1978).

For our investigation we used strains of *Staphylococcus aureus, S. epidermidis, Streptococcus* G, *Strep. agalactiae, Escherichia coli* T18, *Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Haemophilus influenzae.*

A solution of the compound in water ($c = 1$ mg ml^{-1}) was diluted to several concentrations (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256) and 50 μl of liquid culture of organisms was added. Readings of the minimal inhibitory concentrations (MIC) were taken after incubation for 18 h at 37 °C.

Results and Discussion

The comparative antibacterial activities of compounds ($6a_1$–a_3, $6d_1$–d_3, $6e_1$–e_3) are shown in Tables 5 and 6.

To answer question (1) — is this type of substances highly active only against Gram-positive organisms — we observe that the compounds show little if any activity against staphylococci and streptococci, except for compound (**4a**), which displays activity against *S. aureus* and *S. epidermidis*. The compounds have lower, but better activity against the clinically important Gram-negative organisms. Compound (**4a**) shows much higher antibacterial activity than other compounds of the first (cephalothin) or second (cefuroxime) generation cephalosporins. The in vitro activity of this compound is comparable to or even higher than that of cephalothin. Its activity against *K. pneumoniae, Ps. aeruginosa* and *H. influenzae* is also better than that of cephalothin and cefuroxime. The activity against *E. coli* T18 and *P. mirabilis* is similar to that of cephalothin.

The second question we were interested in relates to the influence of substituents at C(3′). Table 6 compares MIC values of compounds in which the 3′-acetoxy group was substituted by a S-nucleophile, i.e. imidazole, 1,2,4-triazole or 1,3,4-thiadiazole.

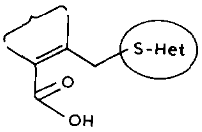
The results indicate that nucleophilic substitution increases the in vitro antibacterial activity of 2,6-difluorobenzoyl- and 2,3,4,5,6-pentafluorobenzoylcephalosporins, whereas it lowers the activity of 2-fluorobenzoylcephalosporins.

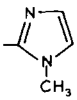
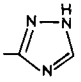
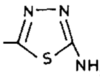
Compounds ($6d_1$–d_3, $6e_1$–e_3) show better antibiotic potency in vitro than the parent compounds (**4d, 4e**) against *E. coli* T18, *Ps. aeruginosa* and *H. influenzae*.

Compound ($6d_1$) shows much higher in vitro antibacterial activity against *E. coli* T18, *Ps. aeruginosa* and *H. influenzae* than the parent compound (**4d**). Against *E. coli* T18 it is 8-times more effective than cephalothin and about 65-times better than cefuroxime. In comparison, the parent compound (**4d**) is 8-fold weaker than cephalothin and similar to cefuroxime as regards

TABLE 6

Antibacterial activity (MIC, μg ml⁻¹) of compounds ($6a_1$–a_3, $6d_1$–d_3, $6e_1$–e_3)



Compound no.	**$6a_1$**	**$6d_1$**	**$6e_1$**	**$6a_2$**	**$6d_2$**	**$6e_2$**	**$6a_3$**	**$6d_3$**	**$6e_3$**	Cephalothin	Cefuroxime
Het											
F	2-F	2,6-$(F)_2$	2,3,4,5,6-$(F)_5$	2-F	2,6-$(F)_2$	2,3,4,5,6-$(F)_5$	2-F	2,6-$(F)_2$	2,3,4,5,6-$(F)_5$		
S. aureus	–	–	–	–	–	–	–	–	–	500	–
S. epidermidis				–	500	–	–	–	–	500	–
Streptococcus G				–	$\boxed{62.5}$	500	–	500	500	500	500
Strep. agalactiae				–	500	500	–	250	–	125	125
E. coli T18	500	$\boxed{7.8}$	250	125	$\boxed{7.8}$	250	250	500	–	62.5	500
P. mirabilis	–	–	–	–	–	–	–	–	–	125	62.5
K. pneumoniae	–	–	–	500	500	–	–	500	500	500	500
Ps. aeruginosa	–	$\boxed{7.8}$	500	500	–	500	250	250	500	500	500
H. influenzae	–	$\boxed{62.5}$	$\boxed{62.5}$	500	–	$\boxed{62.5}$	–	$\boxed{62.5}$	250	250	250

activity. Compound (**6e$_1$**) shows 2-fold better in vitro activity against *E. coli* T18 and much better in vitro activity against *H. influenzae*.

Compounds (**6d$_1$–d$_2$, 6e$_1$–e$_2$**) show better antibiotic potency in vitro than their parent compounds (**4d, 4e**) against *E. coli* T18.

Compound (**6d$_2$**) displays very good in vitro antibacterial activity against *Strep.* G and *E. coli* T18. Compounds (**6e$_1$–e$_2$**) have good in vitro activity against *H. influenzae*. Against *Strep.* G compound (**6d$_2$**) is 8-times better than cephalothin or cefuroxime. Against *E. coli* T18 compound (**6e$_2$**) is 8-times better than cephalothin and twice as effective as cefuroxime. Compound (**6e$_2$**) is 4-times better than cephalothin or cefuroxime against *H. influenzae*.

Compounds (**6d$_3$**) and (**6e$_3$**) show better antibiotic potency in vitro than their parent compounds (**4d, 4e**) against *H. influenzae*. Compound (**6d$_3$**) is twice as effective as the parent compound (**4d**) against *H. influenzae* and 4-times more active than cephalothin or cefuroxime, but compound (**6e$_3$**) is more active against *K. pneumoniae* and *H. influenzae*. Its in vitro activity is similar to that of cefuroxime.

Finally, the in vitro antibacterial activity depends on the heterocyclic ring at C(3′), as follows: imidazole > 1,3,4-thiadiazole > 1,2,4-triazole.

The most surprising result of this study concerns compound (**4a**) which exhibits in vitro antibacterial activity especially against staphylococci, *E. coli* T18 and *H. influenzae*.

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