SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF CEPHALOSPORINS WITH A 1-PYRIDINIUM SUBSTITUENT CARRYING A 5-MEMBERED HETEROCYCLE AT THE C-3 POSITION

AKIO EJIMA, TAKESHI HAYANO, TSUTOMU EBATA, TAKAYASU NAGAHARA, HIROKO KŌDA, HIROAKI TAGAWA and MINORU FURUKAWA

Research Institute, Daiichi Seiyaku Co., Ltd.,
1-16-13 Kitakasai, Edogawa-ku, Tokyo 134, Japan

(Received for publication July 12, 1986)

A series of potent antimicrobial agents have been prepared. These derivatives are cephalosporins carrying a pyridine ring substituted with a heterocycle in the C-3 position. Some of them showed excellent activity not only against Gram-negative organisms including *Pseudomonas aeruginosa* but also against Gram-positive ones. In view of their biological and physico-chemical properties, 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[4-(2 or 5-oxazolyl)-1-pyridinium]methyl-3-cephem-4-carboxylate **8f** (DQ-2522) and **8g** (DQ-2556) were chosen as candidates for further evaluation.

Many cephalosporins with a 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido substituent at the C-7 position of the cephem nucleus show excellent antimicrobial activity, such as cefotaxime[1] (**1**, CTX), cefmenoxime[2] (**2**, CMX), ceftizoxime[3] (**3**, CZX) and ceftriaxone[4] (**4**, CTRX). However their activity against *Pseudomonas aeruginosa* is relatively weak. On the other hand, cefsulodin[5] (**5**, CFS) and ceftazidime[6] (**6**, CAZ) which are reported to be effective against pseudomonal infections bear a 1-pyridinium group at the C-3 position.

These observations prompted the authors to prepare a new series of cephalosporins with 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido and 1-pyridinium groups at the C-7 and C-3 positions, respectively.

In this report, we describe the synthesis, physico-chemical properties and antimicrobial activities of cephalosporins with 5-membered heterocyclic rings such as imidazole, oxazole and pyrazole *etc.* in the pyridinium group (**8a**~**8j**), as shown in Scheme 1.

Chemistry

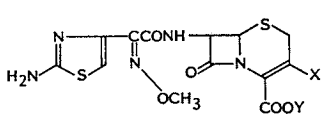
Pyridine derivatives each substituted with a 5-membered heterocyclic ring (**7a**~**7j**) were prepared according to the literature[7~13]. The desired compounds (**8a**~**8j**) were prepared by reacting the above pyridine derivatives with 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid or its salt (**1**) in the presence of NaI. In the reaction of **7b** with **1**, besides the desired compound (**8b**), its isomer (**9**) was obtained.

Some typical compounds were crystallized from aqueous sulfuric acid solution to give crystals of their sulfate salts, all of which, except the crystals of **8i**, were found to have good stability, as shown in Table 2.

Biological Results and Discussion

The minimum inhibitory concentrations (MICs) of the prepared cephalosporins (**8a**~**8j**) are given in Table 1.

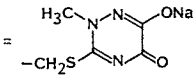
Fig. 1.

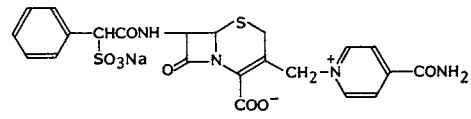


Cefotaxime (**1**, CTX) X = CH_2OAc Y = Na

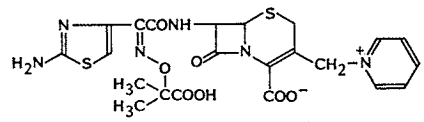
Cefmenoxime (**2**, CMX) X = $-CH_2S$  Y = H

Ceftizoxime (**3**, CZX) X = H Y = Na

Ceftriaxone (**4**, CTRX) X =  Y = Na

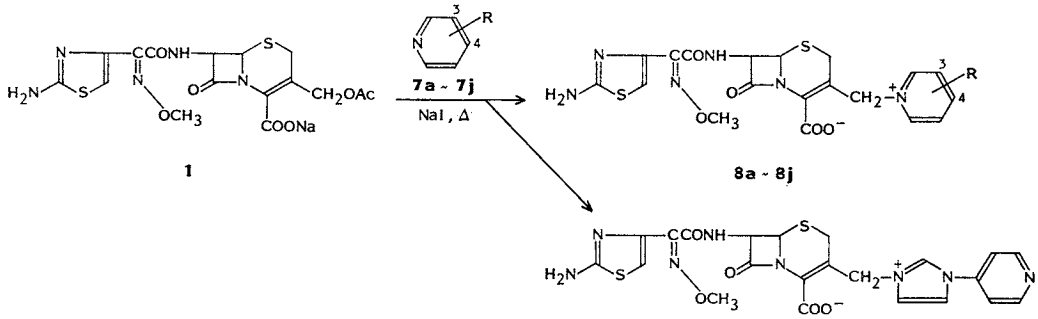


Cefsulodin (**5**, CFS)



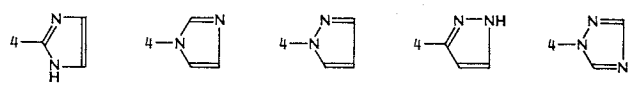
Ceftazidime (**6**, CAZ)

Scheme 1.

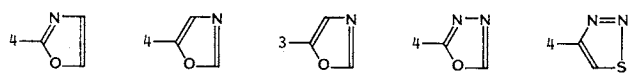


1 **8a ~ 8j** **9**

R:



a **b** **c** **d** **e**



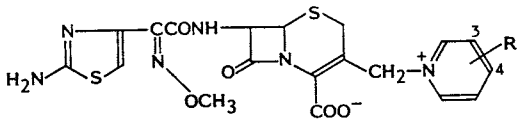
f **g** **h** **i** **j**

These cephalosporins are as active against the Gram-negative organisms, *i.e.*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae* as CTX and CAZ; against *Citrobacter freundii* and *Serratia marcescens*, they are more active than CTX and CAZ; and against *Pseudomonas aeruginosa*, their activities are 4~8 times greater than that of CTX, and similar to that of CAZ.

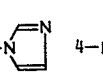
Moreover, against Gram-positive organisms, these compounds are more active than CTX and CAZ. Against *Staphylococcus aureus* and *Bacillus subtilis*, most of them are 4~8 times more active than CTX and 16~32 times more active than CAZ. However, like CTX and CAZ the compounds are not active against *Streptococcus faecalis*.

In a comparison of **8g** and **8h**, the difference of the substitution position of the heterocyclic ring

Table 1. Antimicrobial activities (MIC, μg/ml) of cephalosporins (**8a~8j**) and their solubilities in H_2O.



8a ~ 8j

	8a*	**8b**	**8c****	**8d***	**8e****	**8f**	**8g**	**8h**	**8i**	**8j**	CTX	CAZ
R:												
Staphylococcus aureus 209P	0.78	0.39	0.39	0.19	1.56	0.78	0.39	0.39	0.39	0.39	3.13	12.5
Streptococcus faecalis ATCC 19433	12.5	12.5	12.5	25	25	25	12.5	12.5	12.5	6.25	25	>100
Bacillus subtilis ATCC 6633	1.56	0.78	1.56	0.78	6.25	1.56	0.78	1.56	3.13	0.39	12.5	50
Escherichia coli NIHJ	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Proteus mirabilis IFO 3849	<0.10	0.10	0.10	<0.10	<0.10	<0.10	<0.10	0.10	0.19	<0.10	<0.10	<0.10
P. vulgaris 08602	<0.10	0.10	<0.10	<0.10	0.10	<0.10	<0.10	0.19	0.10	<0.10	<0.10	<0.10
Citrobacter freundii IID976	<0.10	0.10	<0.10	<0.10	0.10	<0.10	<0.10	<0.10	0.10	0.10	0.20	0.39
Enterobacter cloacae 03400	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.10	<0.10	<0.10	0.10
Serratia marcescens 10100	<0.10	<0.10	<0.10	0.19	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.20	0.20
Pseudomonas aeruginosa 32104	25	1.56	3.13	1.56	1.56	3.13	1.56	3.13	0.78	1.56	12.5	1.56
Solubility[a] in H_2O at pH 6.0~7.0 (W/V %)	<5	>10	<5	<5	<5	>20	>20	>20	>20	<5	>20	>20

[a] Each compound was dissolved in H_2O and the solution was adjusted to pH 6.0~7.0 with Na_2CO_3 at 23°C.

* 3HCl salt.

** 2HCl salt.

Table 2. Physico-chemical properties and acute toxicity of **8b**, **8f**, **8g** and **8i**.

	8b	**8f**	**8g**	**8i**
Stability in H_2O at 23°C for 8 hours (% of initial)	>90	>90	>90	>90
Stability of crystals (H_2SO_4 salt) at 60°C for 72 hours (% of initial)	NT	90	90	50
Acute toxicity in mice, iv (LD_{50}; g/kg)	~4	>4	>4	>4

NT: Not tested.

on the pyridine moiety showed no effect on antimicrobial activity.

On the other hand, a heterocyclic ring on the pyridine was observed to influence the solubility in water, which is a very important factor in the selection of an injectable candidate. As shown in Table 1, compounds **8c**, **8d**, **8e** and **8j** (with pyrazole, triazole and thiadiazole) showed low solubility, whilst **8b**, **8f**, **8g** and **8i** (with imidazole, oxazole and oxadiazole) had good solubility.

In view of their activity and solubility, four compounds, **8b**, **8f**, **8g** and **8i**, were selected, and their LD_{50}s and stabilities were examined (Table 2).

Compound **8b** was more toxic than the other three compounds, and the crystals of the sulfate of **8i** were less stable than those of **8f** and **8g**.

Accordingly, compounds **8f** (DQ-2522) and **8g** (DQ-2556), which have an oxazole ring on the pyridine, were chosen as candidates for further evaluation. These results have been submitted for publication elsewhere.

Experimental

FT-NMR spectra were recorded at 200 MHz on a Varian XL-200 spectrometer. The signals were listed in δ values using 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. IR spectra were obtained using Hitachi Models 260-30 and 270-30. Melting points were determined on a Yanagimoto apparatus and are uncorrected.

Quantitative analysis of compounds **8b**, **8f**, **8g** and **8i** were performed by reversed-phase HPLC using YMC-Pack A-312 (Yamamura Chemical Laboratory Co., Ltd.) as the stationary phase, H_2O - CH_3CN - CH_3COOH - triethylamine, 400 : 60 : 2 : 1 or 400 : 50 : 4 : 1 as the mobile phase, and *p*-aminobenzoic acid or sodium benzenesulfonate as an internal standard.

Measurement of *In Vitro* Antibacterial Activity

MICs were measured according to the 2-fold broth dilution method using Mueller-Hinton broth (Difco Laboratories, Detroit, Mich., U.S.A.). The inoculum size was about 10^5 cfu/ml. The MIC was defined as the lowest concentration which prevented visual growth of bacteria after incubation at 37°C for 18 hours.

General Preparation of 7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3 or 4-(5-membered heterocyclic)-1-pyridinium]methyl-3-cephem-4-carboxylate (**8a**~**8j**)

Sodium 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylate (1 mmol), NaI (10 mmol) and the pyridine derivative (2.5 mmol) were added to a mixture of 0.3 ml of 4 N HCl and 0.9 ml of CH_3CN and stirring at 80°C for 1 hour in an atmosphere of N_2.

After being allowed to cool, the reaction mixture was poured into acetone and the precipitate formed was separated by filtration and washed with acetone.

The resulting crude powder was dissolved in water, and the solution was subjected to column chromatography on Diaion HP-20. The column was developed with a mixture of H_2O - THF. The eluate was further subjected to reversed-phase HPLC using Develosil ODS-5 (Nomura Kagaku Co., Ltd.) and developed with a mixture of H_2O - CH_3CN or a mixture of H_2O - CH_3CN adjusted to pH 3

Table 3. Physical data of cephalosporins (**8a**~**8j**).

Compound No.	Formula*	MP (dec, °C)	IR (KBr) β-lactam
8a	$C_{22}H_{20}N_8O_5S_2 \cdot 3HCl \cdot 2\frac{1}{2}H_2O$	165~175	1770
8b	$C_{22}H_{20}N_8O_5S_2 \cdot 2H_2O$	160~165	1770
8c	$C_{22}H_{20}N_8O_5S_2 \cdot 2HCl \cdot 2H_2O$	165~175	1780
8d	$C_{22}H_{20}N_8O_5S_2 \cdot 3HCl \cdot 2H_2O$	165~175	1775
8e	$C_{21}H_{19}N_9O_5S_2 \cdot 2HCl \cdot 2H_2O$	155~170	1770
8f	$C_{22}H_{19}N_7O_6S_2 \cdot 2H_2O$	160~170	1770
8g	$C_{22}H_{19}N_7O_6S_2 \cdot 1\frac{1}{2}H_2O$	145~155	1765
8h	$C_{22}H_{19}N_7O_6S_2 \cdot 1\frac{1}{2}H_2O$	160~170	1765
8i	$C_{21}H_{18}N_8O_6S_2 \cdot 2\frac{1}{2}2H_2O$	160~170	1770
8j	$C_{21}H_{18}N_8O_5S_3 \cdot 2\frac{1}{2}H_2O$	175~185	1770

* All compounds were analyzed for C, H and N. Analytical results were within $\pm 0.4\%$ of calculated values.

Table 4. ^1H NMR (D_2O, δ) data of cephalosporins (**8a**~**8j**).

Compound No.	C(6)-H	C(7)-H	N-OCH_3	Pyridine ring-H	5-Membered ring-H
8a	5.35	5.88	4.07	8.57, 9.24	7.85
8b	5.32	5.81	3.91	8.21, 9.07	7.33, 8.61
8c	5.35	5.91	4.06	8.35, 8.99	6.83, 8.10, 8.60
8d	5.36	5.91	4.06	8.42, 8.95	7.19, 7.97
8e	5.36	5.89	4.05	8.53, 9.16	8.43, 9.51
8f	5.33	5.84	3.94	8.24, 9.02	8.16, 8.55
8g	5.32	5.79	3.91	8.55, 9.14	7.61, 8.26
8h	5.31	5.83	3.96	8.17, 8.85, 8.99, 9.50	7.88, 8.43
8i	5.33	5.81	3.93	8.69, 9.33	9.30
8j	5.36	5.91	4.06	8.77, 9.15	9.96

Table 5. Physical data of sulfate salts of cephalosporins **8b**, **8f**, **8g** and **8i**.

	8b	**8f**	**8g**	**8i**
MP (°C, dec)	200	210	200	180
Formula*	$C_{22}H_{20}N_8O_5S_2 \cdot H_2SO_4 \cdot 3H_2O$	$C_{22}H_{19}N_7O_6S_2 \cdot H_2SO_4 \cdot \frac{1}{2}H_2O$	$C_{22}H_{19}N_7O_6S_2 \cdot H_2SO_4 \cdot 2H_2O$	$C_{21}H_{18}N_8O_6S_2 \cdot H_2SO_4 \cdot 1\frac{1}{2}H_2O$
IR (KBr) cm^{-1}, β-lactam	1795	1795	1785	1790

* All compounds were analyzed for C, H and N. Analytical results were within $\pm 0.5\%$ of calculated values.

with dilute HCl.

The combined eluate was concentrated *in vacuo* and the residue was triturated with Et_2O to obtain the title compounds. The physical data of the products are showed in Tables 3 and 4.

In the case of the reaction of **7b** and **1**, in addition to the desired compound (**8b**), 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-(4-pyridyl)-1-imidazolinium]methyl-3-cephem-4-carboxylate (**9**) was obtained as a by-product after purification by reversed-phase HPLC.

MP 155~165°C (dec); IR (KBr) cm^{-1} 1780 (β-lactam); ^1H NMR (D_2O) δ 4.03 (3H, s, methoxy), 5.35 (1H, d, J=5 Hz, C(6)-H), 5.89 (1H, d, J=5 Hz, C(7)-H), 8.01 (1H, s, imidazole-H), 8.33 (1H, s, imidazole-H), 8.40 (1H, d, J=6 Hz, pyridine-H), 9.11 (1H, d, J=6 Hz, pyridine-H), 9.96 (1H, s, imidazole-H).

Anal Calcd for $C_{22}H_{20}N_8O_5S_2 \cdot 3HCl \cdot 2\frac{1}{2}H_2O$: C 38.02, H 4.06, N 16.12.
Found: C 38.09, H 4.08, N 16.16.

Crystallization of Sulfate Salt

Compound **8b**, **8f**, **8g** or **8i** (7 g) was dissolved in 25 ml of H_2O, and 13 ml of 2 N H_2SO_4 was added thereto. The mixture was allowed to stand for about 30 minutes to give crystals of 7β-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[4-(5-membered ring)-1-pyridinium]methyl-3-cephem-4-carboxylate sulfate (4 g). The physical data and stabilities of the resulting substances are shown in Table 5.

Acknowledgment

The authors wish to thank Drs. Y. OSADA and T. UNE for their helpful discussion in preparing this paper, and Mr. T. FUJIMOTO and Mrs. Y. TAKAHASHI for their antimicrobial testing.

References

1) NEU, H. C.; N. ASWAPOKEE, P. ASWAPOKEE & K. P. FU: HR-756, a new cephalosporin active against gram-positive and gram-negative aerobic and anaerobic bacteria. Antimicrob. Agents Chemother. 15: 273~281, 1979
2) OCHIAI, M.; O. AKI, A. MORIMOTO, T. OKADA & Y. MATSUSHITA: New cephalosporin derivatives with high antibacterial activities. Chem. Pharm. Bull. 25: 3115~3117, 1977
3) TAKAYA, T.; H. TAKASUGI, T. MASUGI, T. CHIBA, H. KOCHI, T. TAKANO & H. NAKANO: Structure-activity relationships of sodium 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (ceftizoxime) and its related compounds. Nippon Kagaku Kaishi 5: 785~804, 1981
4) PEINER, R.; U. WEISS, U. BROMBACHER, P. LANZ, M. MONTAVON, A. FURLENMEIER, P. ANGEHRN & P. J. PROBST: Ro 13-9904/001, a novel potent and long-acting parenteral cephalosporin. J. Antibiotics 33: 783~786, 1980
5) NOMURA, H.; T. FUGONO, T. HITAKA, I. MINAMI, T. AZUMA, S. MORIMOTO & T. MASUDA: Semisynthetic β-lactam antibiotics. 6. Sulfocephalosporins and their antipseudomonal activities. J. Med. Chem. 17: 1312~1315, 1974
6) O'CALLAGHAN, C. H.; P. ACRED, P. B. HARPER, D. M. RYAN, S. M. KIRBY & S. M. HARDING: GR 20263, a new broad-spectrum cephalosporin with anti-pseudomonal activity. Antimicrob. Agents Chemother. 17: 876~883, 1980
7) BAUER, V. J.; G. E. WIEGAND, W. J. FANSHAWE & S. R. SAFIR: Hypoglycemic quaternary azolylpyridinium salts. Inactive analogs. J. Med. Chem. 12: 944~945, 1969
8) KHAN, M. A. & J. B. POLYA: Syntheses of heterocyclic compounds. Part II. *N*-Arylazoles by ullmann condensation. J. Chem. Soc. (C) 1970: 85~91, 1970
9) BAUER, J. B.; H. P. DALALIAN, W. J. FANSHAWE & S. R. SAFIR: 4-[3(5)-Pyrazolyl]pyridinium salts. A new class of hypoglycemic agents. J. Med. Chem. 11: 981~984, 1968
10) DADKHAH, M. & B. PRIJS: Über Pyridyloxazole, eine neue Klasse Dipyridyl-ähnlicher Verbindungen. Helv. Chim. Acta 45: 375~381, 1962
11) SAIKACHI, H.; T. KITAGAWA, H. SASAKI & A. M. VAN LEUSEN: Synthesis of furan derivatives. LXXXV. Condensation of heteroaromatic aldehydes with tosylmethyl isocyanide. Chem. Pharm. Bull. 27: 793~796, 1976
12) RUNTI, C.; L. SINDELLARI & C. NISI: Reactions between organic nitrogen compounds and ethyl orthoformates. I. Hydrazides and derivatives. Ann. Chim. (Rome) 49: 1649~1667, 1959
13) LALEZARI, I.; A. SHAFIEE & S. YAZDANY: Selenium heterocycles X: Synthesis and antibacterial activity of pyridyl-1,2,3-thiadiazoles and pyridyl-1,2,3-selenadiazoles. J. Pharm. Sci. 63: 628~629, 1974