Synthetic Cephalosporins. VI.[1] Synthesis and Antibacterial Activity of 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-(3-hydroxy-4-pyridon-1-yl)methyl-3-cephem-4-carboxylic Acid and Related Compounds

Kenji SAKAGAMI,*,[a] Katsuyoshi IWAMATSU,[a] Kunio ATSUMI[a] and Minoru HATANAKA[b]

Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.,[a] Morooka-cho, Kohoku-ku, Yokohama 222, Japan and the Institute of Scientific and Industrial Research, Osaka University,[b] Mihogaoka, Ibaraki, Osaka 567, Japan. *Received November 29, 1989*

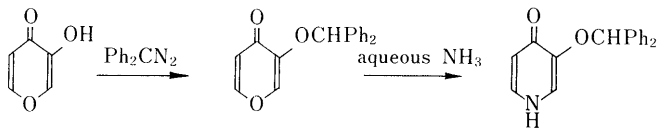
Synthesis and antibacterial activity of 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-(3-hydroxy-4-pyridon-1-yl)-3-cephem-4-carboxylic acid (12) and its related compounds are described. Compound 12 exhibited excellent antibacterial activity against gram-negative bacteria, and its anti-pseudomonal activity was ten to fifteen times greater than that of ceftazidime.

Keywords cephalosporin; anti-pseudomonal activity; structure–activity relationship; 3-hydroxy-4-pyridone

The cephalosporins bearing 2-alkoxyimino 2-(2-aminothiazol-4-yl)acetamido moieties at the C-7 side chain have been widely used for bacterial chemotherapy. Representative are cefotaxime,[2] ceftizoxime,[3] cefmenoxime,[4] ceftriaxone[5] and ceftazidime[6] which have a broad and potent antibacterial activity against gram-positive and gram-negative bacteria. However, most of them have weak activity against *Pseudomonas aeruginosa*, except for ceftazidime. In the course of studies on the development of new semi-synthetic cephalosporins, we found that 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-(3-hydroxy-4-pyridon-1-yl)methyl-3-cephem-4-carboxylic acid (**12**) showed an excellent antibacterial activity against gram-negative bacteria, especially against *Pseudomonas aeruginosa*. Herein reported is the synthesis and structure–activity relationship of **12** and its related compounds.

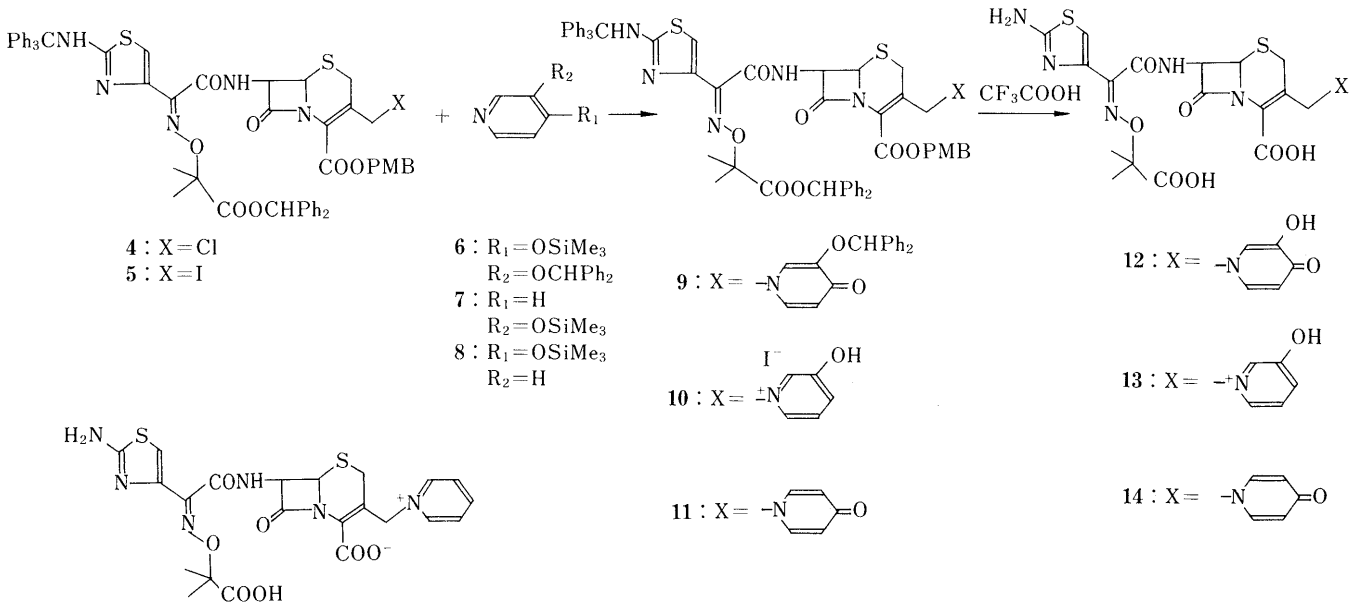
Results and Discussion

Chemistry 3-Diphenylmethoxy-4-pyridone (**3**), required as a new side chain of C-3 position of cephalosporin was prepared from 3-hydroxy-4-pyrone **1**.[7] Compound **1** was treated with diphenyldiazomethane to give 3-diphenylmethoxy-4-pyrone (**2**) in 84% yield, which was converted into **3** by treatment with concentrated ammonia solution in 69% yield. *p*-Methoxybenzyl 7-[(*Z*)-2-(2-tritylaminothiazol-4-yl)-2-(1-diphenylmethoxycarbonyl-1-methyl)ethoxyiminoacetamido]-3-chloromethyl-3-cephem-4-carboxylate (**4**) was prepared from *p*-methoxybenzyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate[8] and (*Z*)-2-(1-diphenylmethoxycarbonyl-1-methylethoxyimino)-2-(2-tritylaminothiazol-4-yl)acetic acid[9] using $POCl_3$ as a coupling reagent and



1 2 3

Chart 1



PMB: *p*-methoxybenzyl group

Chart 2

TABLE I. *In Vitro* MICs (μg/ml)

Test organisms	12	13	14	Ceftazidime
Staphylococcus aureus 606	25	25	25	6.25
S. aureus SMITH (1)	25	25	25	3.13
Escherichia coli W3630 RGN823	0.39	1.56	3.13	0.39
E. coli No. 29	0.10	0.78	0.78	0.20
Klebsiella pneumoniae GN69	0.05	0.39	0.39	0.10
K. pneumoniae PCI602	<0.025	0.39	0.78	0.20
Salmonella typhi 0-901-W	<0.025	0.39	0.39	0.05
S. enteritidis No. 11	<0.025	0.10	0.20	0.05
Escherichia coli 255	6.25	50	>100	12.5
Proteus vulgaris GN76	<0.025	0.05	<0.025	0.05
P. vulgaris GN76/C-1/S-1	<0.025	<0.025	<0.025	0.05
Citrobacter freundii GN346	6.25	>100	>100	50
Enterobacter cloacae GN7471	6.25	50	50	3.13
E. cloacae G-0008	0.39	0.78	1.56	0.20
Serratia marcescens GN10857	0.39	12.5	25	0.78
S. marcescens No. 1	0.05	0.78	1.56	<0.025
Pseudomonas aeruginosa GN10362	0.39	12.5	25	1.56
P. aeruginosa E-2	<0.025	12.5	25	0.78
P. aeruginosa IAM-1007	<0.025	6.25	25	0.78
P. aeruginosa ML Rms139	<0.025	6.25	12.5	0.78

was converted into the iodide **5** by treatment with NaI in acetone.

Displacement reaction of the 3-iodomethyl compound (**5**) with pyridone **3** was readily performed by the following one-pot procedure. Pyridone **3** was first treated with bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in ethyl acetate to generate 3-diphenylmethoxy-4-trimethylsilyloxypyridine (**6**), which readily reacted with **5** at room temperature to afford the corresponding pyridinium iodide. Aqueous work-up of the reaction mixture furnished **9** in 64% overall yield.

In a similar way, 3-hydroxypyridine and 4-pyridone were allowed to react with **5** *via* the trimethylsilyl ethers **7** and **8** to give **10** and **11**, respectively. Removal of the protective groups of **9**, **10** and **11** with CF_3COOH afforded the new cephalosporins **12**, **13** and **14**, respectively.

Antibacterial Activity The minimum inhibitory concentrations (MICs) of the new cephalosporins were determined by the standard, two-fold, agar-dilution method. In Table I, the MIC values of these compounds against several gram-positive and gram-negative bacteria are summarized and compared with those of ceftazidime.

Compound **12** showed the most potent activity against gram-negative bacteria. In particular, its anti-pseudomonal activity was ten to fifteen times greater than that of ceftazidime. It is also noted that **12** shows potent activity against *Escherichia coli* 255, a cephalosporinase-producing strain. Compounds **13** and **14** bearing single oxo functionality on the pyridino moieties, however, had comparable activity with ceftazidime against gram-negative bacteria, but showed reduced activity against *Pseudomonas aeruginosa*. This implies that the two adjacent oxo functionality on the pyridino moiety of C-3 side chain is essential for potent anti-pseudomonal activity in this series of cephalosporins.

In conclusion, we have found that 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-(3-hydroxy-4-pyridon-1-yl)methyl-3-cephem-4-carboxylic acid (**12**) showed extremely potent anti-pseudomonal activity, as well as broad and excellent activity against gram-negative bacteria. Although **12** showed weak activity against gram-positive bacteria, our results indicate that 3-hydroxy-4-pyridon-1-yl methyl group is a potent substituent in the 3-position of cephalosporin.

Experimental

Melting points were uncorrected. Infrared (IR) spectra were recorded on a JASCO-IR-1 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined with tetramethylsilane as an internal standard on either a Hitachi R-90H or JAXC 400GX, chemical shifts being given in ppm unit. Mass spectra (MS) were taken on a Hitachi M-80B mass spectrometer.

3-Diphenylmethoxy-4-pyrone (2) A solution of 3-hydroxy-4-pyrone **1** (5 g) in methanol (50 ml) was treated with diphenyldiazomethane (18 g). The mixture was stirred for 2 h at room temperature and concentrated *in vacuo* to give the crystalline residue, which was recrystallized from methanol–isopropyl ether to afford **2** (10.5 g) as colorless crystals. mp 131—132 °C. IR (Nujol): 1620, 1550, 1530 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.30 (1H, d, J=6.4 Hz, 5-H), 6.38 (1H, s, C<u>H</u>Ph_2), 7.15—7.55 (12H, m, arom, 2-H, 6-H). Electron impact-mass spectra (EI-MS) *m/z*: 278 (M^+). *Anal.* Calcd for $C_{18}H_{14}O_3$: C, 77.68; H, 5.07. Found: C, 77.60; H, 4.86.

3-Diphenylmethoxy-4-pyridone (3) A solution of **2** (1 g) in methanol (3 ml) was treated with a concentrated ammonia solution (10 ml) at room temperature, and the mixture was allowed to stand for 72 h. After evaporation of the solvent, the residue was partitioned between dichloromethane (20 ml) and water (10 ml). The organic layer was washed twice with water, dried and evaporated *in vacuo* to give a pale yellow powder (684 mg) of **3**. mp>200 °C. IR (Nujol): 1620, 1550, 1530 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.15 (1H, d, J=6.4 Hz, 5-H), 6.58 (1H, s, C<u>H</u>Ph_2), 7.15—7.55 (12H, m, arom, 2-H, 6-H). EI-MS *m/z*: 277 (M^+). *Anal.* Calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 77.75; H, 5.38; N, 5.28.

***p*-Methoxybenzyl 7-[(*Z*)-2-(2-Tritylaminothiazol-4-yl)-2-(1-diphenylmethoxycarbonyl-1-methyl)ethoxyiminoacetamido]-3-(3-diphenylmethoxy-4-pyridon-1-yl)-3-cephem-4-carboxylate (9)** A mixture of **4** (524 mg) and sodium iodide (150 mg) in acetone (10 ml) was stirred at room temperature for 1 h. After dilution of the mixture with ethyl acetate (50 ml), the entire mixture was washed with water, dried and evaporated *in vacuo* to yield **5**.

A solution of **3** (277 mg) in ethyl acetate (10 ml) was treated with BSTFA (0.22 ml) at room temperature. A solution of crude **5** in ethyl acetate (20 ml) was added to the mixture at room temperature. The reaction mixture was stirred at the same temperature for 3 h, and then poured into cold water (10 ml). After further stirring at 5 to 10 °C for 30 min, the organic layer was washed with water and brine, dried and evaporated *in vacuo*. The remaining residue was chromatographed on silica gel using chloroform–methanol (50 : 1) as an eluent to give a pale yellow powder (413 mg)

of **9**. mp 125—127 °C (dec.). IR (Nujol): 3350, 1780, 1720, 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.65 (3H, s, CH_3), 1.68 (3H, s, CH_3), 2.45, 2.82 (2H, ABq, J=16 Hz, 2-H), 3.75 (3H, s, OCH_3), 4.25, 4.73 (2H, ABq, J=12 Hz, 3′-H), 4.80 (1H, d, J=5.2 Hz, 6-H), 5.15, 5.26 (2H, ABq, J=9 Hz, –CH_2–O–), 5.90 (1H, dd, J=5.2, 8 Hz, 7-H), 6.35—7.45 (47H, m, arom, C<u>H</u>Ph_2, NH). Field desorption-mass spectra (FD-MS) *m/z*: 1273 $(M+H)^+$.

***p*-Methoxybenzyl 7-[(*Z*)-2-(2-Tritylaminothiazol-4-yl)-2-(1-diphenylmethoxycarbonyl-1-methyl)ethoxyiminoacetamido]-3-(3-hydroxy-1-pyridinio)methyl-3-cephem-4-carboxylate Iodide (10)** Using the procedure for the synthesis of **9** described above, this compound was prepared from **5** and 3-hydroxypyridine. IR (Nujol): 1780, 1720, 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.65 (3H, s, CH_3), 1.66 (3H, s, CH_3), 3.00, 3.58 (2H, ABq, J=16 Hz, 2-H), 3.75 (3H, s, OCH_3), 4.85, 5.15 (2H, ABq, J=12 Hz, 3′-H), 4.90 (1H, d, J=5 Hz, 6-H), 5.10 (2H, br s, –CH_2–O–), 5.90 (1H, dd, J=5, 8 Hz, 7-H), 6.60—7.35 (37H, m, arom, C<u>H</u>Ph_2, NH).

***p*-Methoxybenzyl 7-[(*Z*)-2-(2-Tritylaminothiazol-4-yl)-2-(1-diphenylmethoxycarbonyl-1-methyl)ethoxyiminoacetamido]-3-(4-pyridon-1-yl)methyl-3-cephem-4-carboxylate (11)** Using the procedure for the synthesis of **9** described above, this compound was prepared from **5** and 4-pyridone. mp 130—135 °C (dec.). IR (Nujol): 3350, 1782, 1720, 1670, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (3H, s, CH_3), 1.65 (3H, s, CH_3), 2.90, 3.30 (2H, ABq, J=16 Hz, 2-H), 3.85 (3H, s, OCH_3), 4.26, 4.96 (2H, ABq, J=12 Hz, 3′-H), 4.95 (1H, d, J=5 Hz, 6-H), 5.25, 5.32 (2H, ABq, J=12 Hz, –CH_2–O–), 6.00 (1H, dd, J=5, 9 Hz, 7-H), 6.35 (2H, d, J=6 Hz, pyridone 3-H, 5-H), 6.62 (1H, s, C<u>H</u>Ph_2), 6.80—7.40 (34H, m, arom, NH). FD-MS *m/z*: 1091 $(M+1)^+$.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-(3-hydroxy-4-pyridon-1-yl)methyl-3-cephem-4-carboxylic Acid (12) Compound **9** (300 mg) was treated at 0 °C with anisole (1 ml) and CF_3COOH (5 ml). After the mixture was stirred at 0 °C for 1 h, it was triturated in isopropyl ether (100 ml) to give a powder. The powder was dissolved in mixture of water (2 ml) and ethyl acetate (5 ml) and adjusted to pH 7.2 with $NaHCO_3$. The aqueous layer was chromatographed on a column of Dianion HP-20 using water as an eluent. The fractions were collected and lyophilized to give disodium salt (103 mg) of **12** as an amorphous powder. IR (Nujol): 3150—3350, 1760 cm^{-1}. ^{1}H-NMR (D_2O, DOH at 4.82) δ: 1.52 (6H, s, $(CH_3)_2$), 3.20, 3.52 (2H, ABq, J=16 Hz, 2-H), 4.92 (2H, s, 3′-H), 5.25 (1H, d, J=5 Hz, 6-H), 5.85 (1H, d, J=5 Hz, 7-H), 6.62 (1H, d, J=6.4 Hz, pyridone 5-H), 7.00 (1H, s, thiazole 5-H), 7.70 (1H, s, pyridone 2-H), 7.75 (1H, d, J=6.4 Hz, pyridone 6-H). Secondary ion mass spectrometry (SI-MS) *m/z*: 623 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{20}N_6Na_2O_9S_2 \cdot 3H_2O$: C, 39.05; H, 3.87; N, 12.42. Found: C, 39.00; H, 3.61; N, 12.37.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-(3-hydroxy-1-pyridinio)methyl-3-cephem-4-carboxylic Acid (13) Using the procedure for the synthesis of **12** described above, monosodium salt of **13** was prepared from **10**. IR (Nujol): 3150—3300, 1760 cm^{-1}. ^{1}H-NMR (D_2O, DOH at 4.82) δ: 1.52 (6H, s, $(CH_3)_2$), 3.25, 3.60 (2H, ABq, J=16 Hz, 2-H), 5.20, 5.28 (2H, ABq, J=12 Hz, 3′-H), 5.32 (1H, d, J=5 Hz, 6-H), 5.92 (1H, d, J=5 Hz, 7-H), 7.20 (1H, s, thiazole 5-H), 7.5—7.6 (2H, m, pyridine 4-H, 5-H). 7.8—7.95 (2H, m, pyridine 2-H, 6-H). SI-MS *m/z*: 585 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{21}N_6NaO_8S_2 \cdot 4H_2O$: C, 40.24; H, 4.45; N, 12.80. Found: C, 40.06; H, 4.41; N, 12.57.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-(4-pyridon-1-yl)-3-cephem-4-carboxylic Acid (14) Using the procedure for the synthesis of **12** described above, disodium salt of **14** was prepared from **11**. IR (Nujol): 3150—3300, 1760 cm^{-1}. ^{1}H-NMR (D_2O, DOH at 4.82) δ: 1.52 (6H, s, $(CH_3)_2$), 3.25, 3.56 (2H, ABq, J=16 Hz, 2-H), 4.90 (2H, s, 3′-H), 5.28 (1H, d, J=5 Hz, 6-H), 5.88 (1H, d, J=5 Hz, 7-H), 6.62 (2H, d, J=8 Hz, pyridone 3-H, 5-H), 7.10 (1H, s, thiazole 5-H), 7.98 (2H, d, J=8 Hz, pyridone 2-H, 6-H). SI-MS *m/z*: 607 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{20}N_6Na_2O_8S_2 \cdot 5H_2O$: C, 37.93; H, 4.16; N, 12.38. Found: C, 37.87; H, 4.17; N, 12.14.

References and Notes

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