Arch. Pharm. Res. Vol.19, No.4, pp. 411-415, 1996

Synthesis and Biological Activity of C-3 Pyridinylethene-substituted Cephalosporins

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(Received June 28, 1996)

A series of aminothiazolylcephalosporin derivatives (**1a-1c**) having pyridinylethenyl group at C-3 position was prepared starting from phosphonium salt **3** and 2-, 3- or 4-pyridinecarboxaldehyde and the antibacterial activity of these compounds was investigated. Among them, 4-pyridinylethenyl derivative was more active than 2- and 3-pyridinylethenyl derivatives against *Staphylococcus aureus* and *Escherichia coli*.

Key words : Cephalosporins, Antibacterial activity, Pyridinylethenyl, Wittig reaction, Pyridinecarboxaldehyde

INTRODUCTION

Many aminothiazolylcephalosporins exhibiting broad and strong antibacterial activity have been developed by the manipulation of the C-3 position of the cephem nucleus. Recent interest has been focused on heterocyclyl-sulfur or nitrogen substitution at the C-3 position (Kume *et al.*, 1993; Nakayama *et al.*, 1990) to afford new orally active cephalosporins.

In the preceding papers, we (Park *et al.*, 1994; Lee *et al.*, 1996) and Nagano *et al.*, (1991) have reported the synthesis and biological activities of the C-3 heterocyclylcarbon-substituted cephalosporin derivatives represented by the structure **A** as shown in Fig. 1. In continuation with our research on the modification of the C-3 position by the substitution of heterocyclylcarbon, we were interested in the introduction of an ethenyl linkage at the C-3 position of the heterocyclylcarbon-substituted cephalosporin derivatives to provide new cephalosporins represented by the structure **1**. In this paper we wish to describe the synthesis of aminothiazolylcephalosporin derivatives having pyridinylethenyl group at the C-3 position. The effect of the position of pyridinyl-substituent at ethenyl group of the C-3 side chain on the antibacterial activity was also examined.

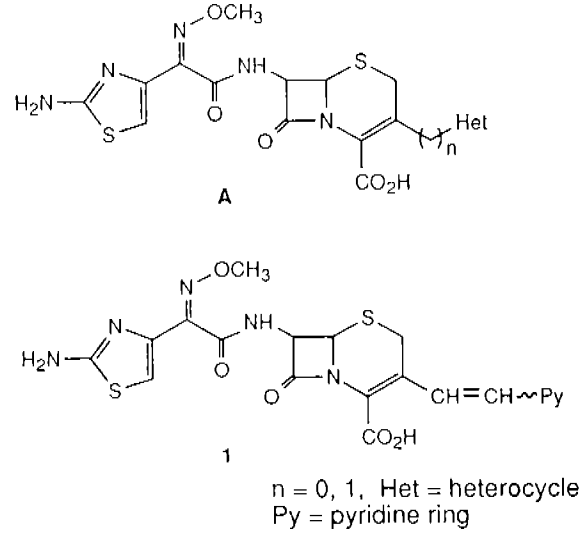

Fig. 1.

MATERIALS AND METHODS

^{1}H NMR spectra were recorded on a Varian gemini-300 300 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. CH_2Cl_2 was distilled from NaH and N,N-dimethylformamide (DMF) was distilled from CaH_2 before using. Flash column chromatography was performed using E. Merck Kieselgel 60 (230-400 mesh) silica gel.

MICs (μg/ml) were determined by the 2-fold agar dilution method using Mueller-Hinton Agar after incubation at 37°C for 18 hours with an inoculum size of 10^7 cfu/ml.

p-Methoxybenzyl 7-phenylacetamido-3-[(*E*)-2-(pyridin-2-yl)ethenyl]-3-cephem-4-carboxylate (4a)

Triphenylphosphine (8.8 g, 33.6 mmol) and sodium

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iodide (5.5 g, 36.7 mmol) was added to a solution of 3-chloromethylcephem (**2**, 15 g, 30.8 mmol) in DMF (40 ml) at 0°C. After stirring at room temperature for 2 h, the reaction mixture was poured into 2-propanol (1 l) to precipitate the salt. The solid was filtered and dissolved in CH_2Cl_2-$CHCl_3$ (1 : 1, 50 ml) and the resulting solid (NaCl and NaI) was removed by filtration. The filtrate was concentrated to 40 ml and poured into diethy ether (200 ml) to obtain a white solid. The solid was filtered, washed with diethyl ether and dried *in vacuo* to give phosphonium iodide **3** (25 g, 97%).

To a solution of triphenylphosphonium iodide (**3**, 200 mg, 0.24 mmol) in CH_2Cl_2 (2 ml) and H_2O (1 ml) was added 2-pyridinecarboxaldehyde (25 mg, 0.23 mmol) and the mixture was adjusted to pH 9 with 10% aqueous Na_2CO_3 solution. After stirring at room temperature for 2 h, the organic layer was separated, washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography (EtOAc/hexane/MeOH=2 : 1 : 1) to afford **4a** (31 mg, 24%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.54, 3.74 (2H, ABq, J=18 Hz, C_2-H), 3.64 (2H, s, $PhCH_2$), 3.79 (3H, s, OCH_3), 4.95 (1H, d, J=5 Hz, C_6-H), 5.19, 5.32 (2H, ABq, J=12 Hz, OCH_2), 5.84 (1H, dd, J=5, 8 Hz, C_7-H), 6.65 (1H, d, J=8 Hz, NH), 6.73 (1H, d, J= 16 Hz, vinyl-H), 6.86-7.56 (12H, m), 7.95 (1H, d, J=16 Hz, vinyl-H), 8.54 (1H, d, J=4 Hz, pyridine 6-H).

p-Methoxybenzyl 7-phenylacetamido-3-[(*Z*)-2-(pyridin-3-yl)ethenyl]-3-cephem-4-carboxylate (4b)

The compound was obtained by a similar procedure using triphenylphosphonium iodide (**3**, 3 g, 3. 57 mmol) and 3-pyridinecarboxaldehyde (420 mg, 3. 92 mmol). Yield; 78% (1.5 g): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.11, 3.27 (2H, ABq, J=18 Hz, C_2-H), 3.60 (2H, s, $PhCH_2$), 3.72 (3H, s, OCH_3), 4.89 (1H, d, J=5 Hz, C_6-H), 5.06, 5.13 (2H, ABq, J=12 Hz, OCH_2), 5. 79 (1H, dd, J=5, 9 Hz, C_7-H), 6.49 (1H, d, J=12 Hz, vinyl-H), 6.52 (1H, d, J=12 Hz, vinyl-H), 6.79-7.79 (12H, m), 8.41 (2H, br s, pyridine 2,6-H).

p-Methoxybenzyl 7-phenylacetamido-3-[(*E*)-2-(pyridin-4-yl)ethenyl]-3-cephem-4-carboxylate (4c)

The compound was obtained by a similar procedure using triphenylphosphonium iodide (**3**, 3 g, 3. 57 mmol) and 4-pyridinecarboxaldehyde (440 mg, 4. 11 mmol). Yield; 86% (1.67 g): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.59, 3.74 (2H, ABq, J=18 Hz, C_2-H), 3.66 (2H, s, $PhCH_2$), 3.81 (3H, s, OCH_3), 5.08 (1H, d, J=5 Hz, C_6-H), 5.18, 5.38 (2H, ABq, J=12 Hz, OCH_2), 5. 85 (1H, dd, J=5, 9 Hz, C_7-H), 6.30 (1H, d, J=9 Hz, NH), 6.59 (1H, d, J=16 Hz, vinyl-H), 7.06-7.38 (11H, m), 7.66 (1H, d, J=16 Hz, vinyl-H), 8.50 (2H, d, J=6 Hz, pyridine 2,6-H).

p-Methoxybenzyl 7-amino-3-[(*E*)-2-(pyridin-2-yl)ethenyl]-3-cephem-4-carboxylate (5a)

To a suspension of PCl_5 (350 mg, 1.67 mmol) in CH_2Cl_2 (5 ml) was added pyridine (0.12 ml, 1.67 mmol) at 0°C under N_2 atmosphere. After stirring for 20 min at room temperature, *p*-methoxybenzyl 7-phenylacetamido-3-[(*E*)-2-(pyridin-2-yl)ethenyl]-3-cephem-4-carboxylate (**4a**, 300 mg, 0.55 mmol) was added in one portion to the mixture at -40°C and stirred at -10°C for 12 h. 1,3-Propanediol (1.5 ml) was addded to the mixture at -20°C and stirred at the same temperature for 5 h and H_2O was added at -5°C. After adding saturated aqueous $NaHCO_3$ solution, the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (10 ml) and the combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography (EtOAc/CH_2Cl_2=1 : 1) to afford a free amine (**5a**, 112 mg, 48%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.64, 3.85 (2H, ABq, J=18 Hz, C_2-H), 3.81 (3H, s, OCH_3), 4.76 (1H, d, J=5 Hz, C_6-H), 4.99 (1H, dd, J=5, 8 Hz, C_7-H), 5.23, 5.37 (2H, ABq, J=12 Hz, OCH_2), 6.85 (1H, d, J=16 Hz, vinyl-H), 6.87-7.62 (7H, m), 7.91 (1H, d, J=16 Hz, vinyl-H), 8.58 (1H, d, J=4 Hz, pyridine 2-H).

p-Methoxybenzyl 7-amino-3-[(*Z*)-2-(pyridin-3-yl)ethenyl]-3-cephem-4-carboxylate (5b)

The compound was obtained by a similar procedure using PCl_5 (0.23 g, 1.1 mmol), pyridine (0.27 ml, 3.7 mmol) and *p*-methoxybenzyl 7-phenylacetamido-3-[(*Z*)-2-(pyridin-3-yl)ethenyl]-3-cephem-4-carboxylate (**4b**, 200 mg, 0.37 mmol). Yield; 78% (125 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.15, 3.36 (2H, ABq, J=18 Hz, C_2-H), 3.80 (3H, s, OCH_3), 4.76 (1H, d, J=5 Hz, C_6-H), 4.94 (1H, dd, J=5, 8 Hz, C_7-H), 5. 14, 5.20 (2H, ABq, J=12 Hz, OCH_2), 6.54 (1H, d, J= 12 Hz, vinyl-H), 6.58 (1H, d, J=12 Hz, vinyl-H), 6.85-7.57 (6H, m), 8.49 (2H, br d, pyridine 2,6-H).

p-Methoxybenzyl 7-amino-3-[(*E*)-2-(pyridin-4-yl)ethenyl]-3-cephem-4-carboxylate (5c)

The compound was obtained by a similar procedure using PCl_5 (0.7 g, 3.35 mmol), pyridine (0.8 ml, 10.02 mmol) and *p*-methoxybenzyl 7-phenylacetamido-3-[(*E*)-2-(pyridin-4-yl)ethenyl]-3-cephem-4-carboxylate (**4c**, 600 mg, 1.11 mmol). Yield; 36% (170 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.60, 3.78 (2H, ABq, J=18 Hz, C_2-H), 3.80 (3H, s, OCH_3), 4.76 (1H, d, J=5 Hz, C_6-H), 4.97 (1H, dd, J=5, 8 Hz, C_7-H), 5.19, 5. 40 (2H, ABq, J=12 Hz, OCH_2), 6.61 (1H, d, J=16 Hz, vinyl-H), 6.91 (2H, d, J=6 Hz, benzene 2,6-H), 7.08

(2H, d, J=5 Hz, pyridine 3,5-H), 7.40 (2H, d, J=6 Hz, benzene 3,5-H), 7.60 (1H, d, J=16 Hz, vinyl-H), 8.50 (2H, d, J=5 Hz, pyridine 2,6-H).

7β-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]-3-[(*E*)-2-(pyridin-2-yl)ethenyl]-3-cephem-4-carboxylic acid trifluoroacetic acid salt (1a)

p-Methoxybenzyl 7-amino-3-[(*E*)-2-(pyridin-2-yl) ethenyl]-3-cephem-4-carboxylate (**5a**, 80 mg, 0.19 mmol) and 2-(aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid S-2-benzothiazolyl ester (**6**, 70 mg, 0.20 mmol) were dissolved in CH_2Cl_2 (2 ml) and stirred at room temperature for 14 h. The reaction mixture was concentrated and purified by flash column chromatography to afford *p*-methoxybenzyl 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]-3-[(*E*)-2-(pyridin-2-yl)ethenyl]-3-cephem-4-carboxylate (85 mg, 74%). This product (50 mg, 0.08 mmol) was dissolved in CH_2Cl_2 (2 ml) and anisole (0.5 ml) and trifluoroacetic acid (1 ml) was added at 0°C. After 30 min, the reaction mixture was concentrated and treated with diisopropyl ether to precipitate **1a** (42 mg, 85%) as trifluoroacetic acid salt. ^{1}H NMR (CD_3OD, 300 MHz) δ 3.78, 3.97 (2H, ABq, J=18 Hz, C_2-H), 4.03 (3H, s, OCH_3), 5.28 (1H, d, J=5 Hz, C_6-H), 5.91 (1H, d, J=5 Hz, C_7-H), 7.00 (1H, s, thiazole-H), 7.01 (1H, d, J=16 Hz, vinyl-H), 7.60 (1H, t, J=8 Hz, pyridine 5-H), 7.93 (1H, d, J=8 Hz, pyridine 3-H), 8.08 (1H, d, J=16 Hz, vinyl-H), 8.20 (1H, d, J=8 Hz, pyridine 4-H), 8.61 (2H, d, J=5 Hz, pyridine 6-H).

7β-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]-3-[(*Z*)-2-(pyridin-3-yl)ethenyl]-3-cephem-4-carboxylic acid trifluoroacetic acid salt (1b)

The compound was obtained by a similar procedure from *p*-methoxybenzyl 7-amino-3-[(*Z*)-2-(pyridin-3-yl)ethenyl]-3-cephem-4-carboxylate (**5b**) in overall 53% yield in two steps. ^{1}H NMR (CD_3OD, 300 MHz) δ 3.61, 3.83 (2H, ABq, J=18 Hz, C_2-H), 4.04 (3H, s, OCH_3), 5.28 (1H, d, J=5 Hz, C_6-H), 5.84 (1H, d, J=5 Hz, C_7-H), 6.70, 6.78 (2H, two d, J=12 Hz, vinyl-H), 7.03 (1H, s, thiazole-H), 7.80-8.69 (5H, m, NH, pyridine).

7β-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetamido]-3-[(*E*)-2-(pyridin-4-yl)ethenyl]-3-cephem-4-carboxylic acid trifluoroacetic acid salt (1c)

The compound was obtained by a similar procedure from *p*-methoxybenzyl 7-amino-3-[(*E*)-2-(pyridin-4-yl)ethenyl]-3-cephem-4-carboxylate (**5c**) in overall 56% yield in two steps. ^{1}H NMR (CD_3OD, 300 MHz) δ 3.75, 3.82 (2H, ABq, J=18 Hz, C_2-H), 4.05 (3H, s, OCH_3), 5.30 (1H, d, J=5 Hz, C_6-H), 5.94 (1H, d, J=5 Hz, C_7-H), 7.02 (1H, s, thiazole-H), 7.09 (1H, d, J=16 Hz, vinyl-H), 8.01 (2H, d, J=6 Hz, pyridine 3, 5-H), 8.10 (1H, d, J=16 Hz, vinyl-H), 8.66 (2H, d, J=6 Hz, pyridine 2,6-H).

RESULTS AND DISCUSSION

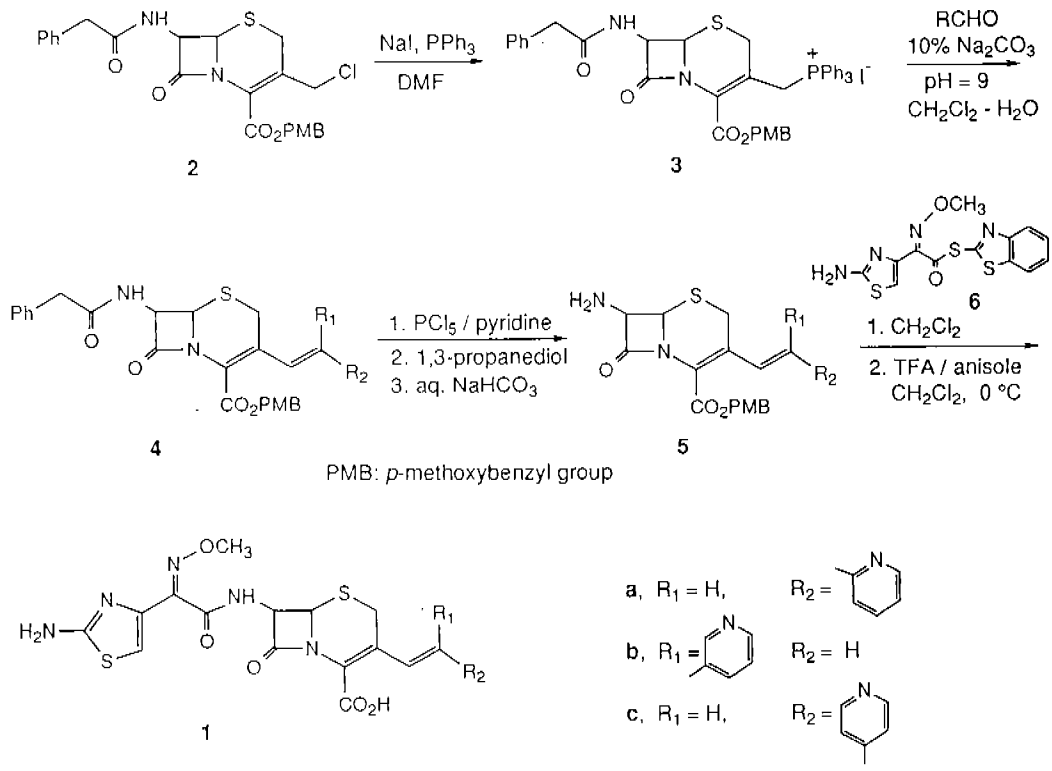
Chemistry

The synthetic route to new aminothiazolylcephalosporin derivatives having pyridinylethenyl group at the C-3 position is illustrated in Scheme 1. 3-Chloromethylcephem **2** (Torii *et al.*, 1982) was treated with NaI and PPh_3 in DMF to give the triphenylphosphonium iodide (**3**) in 97% yield. Wittig reaction of **3** with a series of pyridinecarboxaldehyde was carried out in CH_2Cl_2-H_2O at room temperature in the presence of 10% aqueous Na_2CO_3 solution to afford ethenyl derivatives (**4a~4c**) in 24~86% yield. Wittig reactions of **3** with 2- and 4-pyridinecarboxaldehyde afforded ethenyl derivatives (**4a, 4c**) exclusively in *E* isomer. However, Wittig reaction of **3** with 3-pyridinecarboxaldehyde afforded ethenyl derivative (**4b**) in a form of 4 : 1 mixture of *Z* and *E* isomers judging from the ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **4b** showed each doublet assigned to olefin protons with a coupling constant of 12 Hz, whereas those of **4a** and **4c** showed doublets with a larger coupling constant of 16 Hz, indicating the major component of **4b** is the *Z* isomer and **4a** and **4c** are the *E* isomer of the C-3 side chain. Since it was difficult to separate these isomers by column chromatography, the products were used for next step without separation of isomers. Phenylacetyl group of **4** was removed efficiently by using PCl_5-1,3-propanediol-$NaHCO_3$ system (Aburaki *et al.*, 1983) to give **5a** and **5c** as a pure *E* isomer, respectively, and **5b** as a pure *Z* isomer after purification by silica gel column chromatography. The free amines (**5a~5c**) were converted to new cephalosporins (**1a~1c**) as trifluoroacetic acid salt by acylation with aminothiazole active ester **6** in CH_2Cl_2 followed by the deprotection of *p*-methoxybenzyl protecting group with CF_3CO_2H-anisole.

Antibacterial Activity

The *in vitro* antibacterial activities of new aminothiazolylcephalosporin derivatives (**1a~1c**) having pyridinylethenyl group at C-3 position against selected Gram-positive and Gram-negative bacteria are shown in Table 1. For comparison, the MIC values of cefixime and cefotaxime are listed.

Most of the compounds were less active against all of the organisms tested than cefotaxime. The activities of these compounds were superior to cefixime against *Staphylococcus aureus* and *Escherichia coli*. However, they were less active than cefixime against Gram-negative bacteria.



Scheme 1. Synthesis of pyridinylethenylcephalosporin derivatives (**1a~1c**)

Table I. Antibacterial activity of pyridinylethenylcephalosporins (**1a~1c**)

Test organism	MIC (μg/ml)				
	1a	**1b**	1c	**CFX**	**CTX**
Streptococcus pyogenes A 308	0.025	0.025	0.013	0.007	0.004
Streptococcus pyogenes A 77	0.013	0.004	0.004	0.049	0.004
Streptococcus faecium MD 8b	50	>100	25	100	50
Streptococcus aureus SG 511	12.5	3.13	0.78	25	1.56
Streptococcus aureus 285	12.5	3.13	1.56	50	3.13
Streptococcus aureus 503	6.25	1.56	0.78	50	1.56
Escherichia coli O 55	0.2	0.2	0.098	0.2	0.013
Escherichia coli DC 0	0.78	1.56	0.2	0.78	0.049
Escherichia coli DC 2	0.025	0.049	0.013	0.39	0.004
Escherichia coli TEM	0.39	0.78	0.2	0.78	0.025
Escherichia coli 1507 E	0.78	0.78	0.2	0.39	0.049
Pseudomonas aeruginosa 9027	100	>100	50	>100	12.5
Pseudomonas aeruginosa 1592 E	100	>100	100	>100	12.5
Pseudomonas aeruginosa 1771	25	50	25	12.5	6.25
Pseudomonas aeruginosa 1771 M	1.56	1.56	0.78	0.2	0.049
Salmonella typhimurium	0.78	0.78	1.56	0.098	0.049
Klebsiella oxytoca 1082 E	25	12.5	25	0.39	0.78
Klebsiella aerogenes 1522 E	0.78	0.78	0.39	0.025	0.025
Enterobacter cloacae P 99	>100	>100	>100	>100	>100
Enterobacter cloacae 1321 E	0.2	0.39	0.2	0.013	0.013

CFX: Cefixime, CTX: Cefotaxime

The effect of configuration of ethenyl group on the antibacterial activity is not clear at this point. But, the position of pyridinyl-substituent at ethenyl group of the C-3 side chain seems to play an important role in improving activity against *Staphylococcus aureus* and *Escherichia coli*; 4-pyridinylethenyl derivative (**1c**) was more active than 2- and 3-pyridinylethenyl derivative (**1a, 1b**).

ACKNOWLEDGEMENT

We are grateful to Korea Ministry of Science and Technology for financial support.

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