SEMISYNTHETIC β-LACTAM ANTIBIOTICS

II. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[2-(ACYLAMINO)-2-(2-AMINOTHIAZOL-4-YL)ACETAMIDO]CEPHALOSPORINS

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(Received for publication April 25, 1986)

Cephalosporin derivatives (**I**) substituted with a neutral, acidic or basic amino acid group as the terminal group attached to the 2-amino-2-(2-aminothiazol-4-yl)acetamido side chain at the C-7 position were synthesized, and the effect of each group on antibacterial activity was examined. The derivatives bearing an amidino or guanidino group showed broad spectra of antibacterial activity similar to those of cefotaxime, but they were relatively sensitive to β-lactamases.

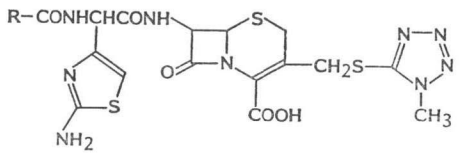
In the preceding paper,[1] the authors reported on the synthesis and antibacterial activity of the cephalosporins having dipeptide side chains at the C-7 position represented by general structure **I** (Fig. 1); the derivatives acylated with neutral amino acid as the second amino acid R showed high antibacterial activity.

In the course of our present research program directed toward the development of new β-lactam antibiotics with enhanced activity, the preparation of cephalosporin derivatives having a 2-amino-2-(2-aminothiazol-4-yl)acetamido side chain acylated with a neutral, acidic or basic group as R at the 7-position of cephem were planned as shown in general structure **I**. This paper described the syntheses and the antibacterial properties of these cephalosporins.

Chemistry

The cephem derivatives listed in Table 1 were prepared by the method shown in Scheme 1. The preparation of α-amino acid derivatives **1** and **2** were reported in the preceding paper.[1]

Fig. 1.

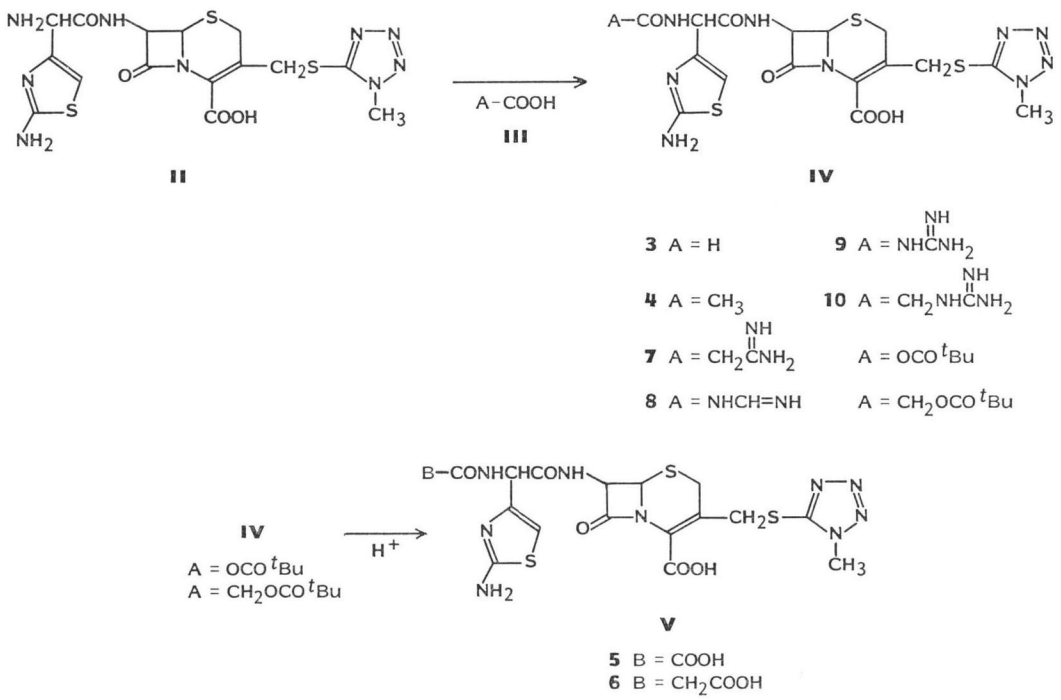


I

Cephem derivatives **3**～**10** were prepared by acylation of 7-amino-3-(1-methyl-1*H*-tetrazol-5-yl)cephem-4-carboxylic acid (7-ATCA) with one of the active derivatives, as shown in Table: a, mixed anhydride; b, acid chloride; c, active ester; and d, acid azide. In the cases of compounds **5** and **6**, the half ester of dicarboxylic acid protected with a *tert*-butyl group was employed as the starting carboxylic acid (**III**), and acylation of the compound **II** with **III** *via* the corresponding active ester gave the protected product (**IV**). Subsequent removal of the *tert*-butyl protecting group was carried out using trifluoroacetic acid-anisole at the final step in the synthetic Scheme 1. This afforded compound **V**.

Amidino or guanidino compounds, **7**～**10**, were obtained by interaction of compound **II** with the starting material (**III**) as acid chloride (or acid azide) in the form of the hydrochloride but without a

Scheme 1.



protecting group. (Refer Experimental section.)

Antibacterial Activity and Discussion

The minimum inhibitory concentration (MIC) values of this series of cephalosporins against selected strains of Gram-positive and Gram-negative bacteria were determined by the standard serial two-fold agar dilution method.[2]

The structure and *in vitro* activity of newly synthesized cephalosporins are listed in Table 1.

Compounds **3** and **4** having a neutral group as the terminal acyl moiety at the 7-position were almost as active or slightly more active against Gram-negative, but less active against Gram-positive bacteria, in comparison with the amino acyl analogs (**1** and **2**) reported in the preceding paper.[1]

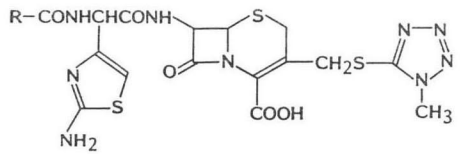
Introduction of an acidic components (**5** and **6**) increased the activity against Gram-negative strains, especially *Proteus vulgaris*, but significantly decreased the activity against Gram-positive strains.

On the other hand, the compounds **7**~**10**, with a strongly basic amidino or guanidino side chain, showed higher activity against Gram-negative bacteria and activity against Gram-positive bacteria that was very similar to that of **1** and **2**. Their antibacterial spectra and activity levels were comparable to those of cefotaxime,[3,4] except in the case of **8**.

The stabilities of representative compounds to β-lactamase were examined. The results, shown in Table 2, indicated that hydroxyimino type derivatives, *e.g.* cefotaxime, are superior to these compounds.

In summary, the cephem derivatives with amidino and guanidino side chains showed high *in vitro* antibacterial activity and expanded spectra comparable to that of cefotaxime. However, further improvement of the stability to β-lactamase is desirable.

Table 1. Structure and antibacterial activity (MIC, μg/ml) of the cephalosporins (**I**).

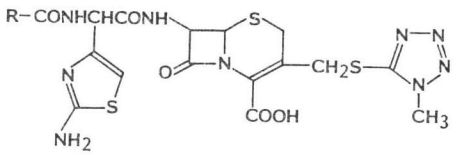


Compound	R	Active derivative*	*S.a.* 209P	*S.a.* Smith	*S.e.*	*E.c.*	*P.v.*	*En.c.*	*S.m.*	*P.a.*
1	CH_2NH_2	c	1.56	1.56	0.78	0.05	100	0.78	1.56	100
2	$CHNH_2$ $\vert$ CH_3	c	1.56	1.56	0.78	0.05	100	0.10	0.78	100
3	H	a	1.56	3.13	3.13	0.05	100	0.10	0.20	50
4	CH_3	b	3.13	3.13	6.25	0.10	100	0.10	0.39	100
5	COOH	c	12.5	12.5	12.5	0.05	3.13	0.10	0.05	6.25
6	CH_2COOH	c	25	12.5	25	0.05	3.13	0.39	0.10	12.5
7	NH $\vert\vert$ CH_2CNH_2	b	0.78	1.56	0.78	0.10	12.5	0.10	0.39	50
8	$CH_2NHCH{=}NH$	b	1.56	1.56	1.56	0.10	100	0.10	0.78	100
9	NH $\vert\vert$ NH_2CNH_2	d	0.78	1.56	0.78	0.05	50	0.10	0.78	12.5
10	NH $\vert\vert$ CH_2NHCNH_2	b	0.78	1.56	0.39	0.05	6.25	0.39	0.20	25
Cefotaxime			1.56	1.56	0.78	0.05	6.25	0.10	0.78	12.5

* a; Mixed anhydride, b; acid chloride, c; active ester, d; acid azide.

Abbreviations: *S.a.* 209P; *Staphylococcus aureus* 209P, *S.a.* Smith; *Staphylococcus aureus* Smith, *S.e.*; *Staphylococcus epidermidis* 7035, *E.c.*; *Escherichia coli* NIHJ, *P.v.*; *Proteus vulgaris* 3167, *En.c.*; *Enterobacter cloacae* 12005, *S.m.*; *Serratia marcescens* 13014, *P.a.*; *Pseudomonas aeruginosa* 2092.

Table 2. Stability against β-lactamase.



Cephalosporinase		Relative rate of hydrolysis					
				R			
Type[a]	Source	CER[b]	CTX[c]	CH_3 | $CHNH_2$ **2**	NH || CH_2CNH_2 **7**	NH || $NHCNH_2$ **9**	NH || CH_2NHCNH_2 **10**
Ia	*E. cloacae* GN7471	100	0.1	0.4	1.0	2.4	3.3
Ib	*E. coli* 1154	100	0.6	0.4	NT	NT	NT
Ic	*P. vulgaris* GN76	100	1.5	34.0	25.3	26.4	60.2
Id	*P. aeruginosa* 2006	100	0.3	0.8	3.2	3.9	8.8

[a] Enzyme classification according to RICHMOND and SYKES.[5]
[b] Cephaloridine. [c] Cefotaxime.
NT: Not tested.

Experimental

Melting points were determined using a Yanagimoto MP-1 micro melting apparatus and are uncorrected. IR spectra were taken on a Hitachi 285 spectrophotometer. NMR spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20B and at 200 MHz on a Varian XL-200 spectrometer using TMS or sodium 2,2-dimethyl-2-silapentane-1-sulfonate (DSS) as an internal standard. Organic solvents were dried over anhydrous Na_2SO_4 and all concentration by evaporation were carried out *in vacuo*.

7β-[DL-2-Formamido-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic Acid (**3**)

Acetic-formic anhydride (0.3 ml) (prepared from formic acid (920 mg) and acetic anhydride (2.04 g) in CH_3CN (20 ml)) was added dropwise to a stirred solution of cephalosporin **II**[5] (968 mg, 2 mmol) and *N,O*-bis(trimethylsilyl)acetamide (2 ml), the mixture being kept cool with ice. After stirring at room temp for 3 hours, the mixture was poured into Et_2O. The precipitates were collected by filtration and dissolved in H_2O and then adjusted to pH 6 with 5% $NaHCO_3$ solution. The aqueous solution was chromatographed on a Diaion HP-20 column. The eluate with 10% aq MeOH was evaporated to remove the MeOH and lyophilized to give the title compound as a powder (360 mg, 35.2%): MP 175~190°C (dec); IR (KBr) 1760, 1660, 1600 cm^{-1}; NMR (D_2O) δ 3.2~4.0 (2H, ABq, C2-CH_2), 4.05 (3H, s, tetrazole-CH_3), 3.9~4.7 (2H, ABq, C3-CH_2), 5.07 and 5.11 (1H, 2×d, J=5 Hz, C6-H), 5.53 (1H, s, thiazole-CHCO), 5.58 and 5.67 (1H, 2×d, J=5 Hz, C7-H), 6.72 and 6.75 (1H, 2×s, thiazole 5-H), 8.22 (1H, s, CHO).

Anal Calcd for $C_{16}H_{17}N_9O_5S_3 \cdot \frac{1}{2}H_2O$: C 36.92, H 3.49, N 24.22.
Found: C 36.93, H 3.40, N 23.82.

7β-[DL-2-Acetamido-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic Acid (**4**)

To a stirred solution of compound **II**[6] (484 mg, 1 mmol), *N,O*-bis(trimethylsilyl)acetamide (1 ml) and propylene oxide (0.5 ml) in CH_3CN (8 ml) was added dropwise acetyl chloride (0.095 ml, 1.2 mmol) with ice-cooling. The mixture was stirred for 1 hour with further ice-cooling, and for another 30 minutes at room temp. The solvent was evaporated off. The residue was added to Et_2O and the precipitates were collected by suction and dissolved in 5% $NaHCO_3$. The solution was adjusted to pH 6 with 5% HCl and subjected to column chromatography (Diaion HP-20). The column was washed

with H_2O and eluted with 5% aq MeOH. The eluate was evaporated to remove MeOH and lyophilized to give the desired compound (17.2 g, 32.7%): MP 172~182°C (dec); IR (KBr) 1760, 1620, 1500 cm^{-1}; NMR (DMSO-d_6) δ 2.09 (3H, s, $COCH_3$), 3.2~3.9 (2H, ABq, C2-CH_2), 3.96 (3H, s, tetrazole-CH_3), 4.0~4.6 (2H, ABq, C3-CH_2), 5.09~5.12 (1H, 2×d, J=5 Hz, C6-H), 5.42 (1H, s, thiazole-CHCO), 5.59 and 5.69 (1H, 2×d, J=5 Hz, C7-H), 6.45 and 6.47 (1H, 2×s, thiazole 5-H).

Anal Calcd for $C_{17}H_{19}N_9O_5S_3$: C 38.85, H 3.64, N 23.99.
Found: C 38.64, H 3.62, N 23.72.

7β-[DL-2-Oxamido-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic Acid (**5**)

1) Preparation of Mono *tert*-Butyl Oxalate: The mixture of *tert*-butyl ethyl oxalate[7] and 1 N NaOH solution was stirred for 30 minutes at room temp, poured into H_2O, and washed with Et_2O. The aqueous layer was made acidic with 10% citric acid and extracted with EtOAc and dried, Evaporation of the solvent afforded the residue as an oil: IR (neat) 1730 cm^{-1}; NMR ($CDCl_3$) δ 1.50 (9H, s, *tert*-Bu).

2) Preparation of **5**: An ice-cooled mixture of mono *tert*-butyl oxalate (730 mg, 5 mmol), *N*-hydroxysuccinimide (633 mg, 5.5 mmol) and *N*,*N*-dicyclohexylcarbodiimide (1.03 g, 5 mmol) in THF (30 ml) was stirred for 3 hours, and then filtered to remove the precipitate formed. The resulting filtrate was added to an ice-cooled solution of cephalosporin **II** (1.21 g, 2.5 mmol) and triethylamine (505 mg, 5 mmol) in THF (30 ml), and the solution was stirred for 2 hours. After the removal of THF from the mixture, the residue was dissolved with 5% $NaHCO_3$ solution and extracted with EtOAc. The organic layer was acidified with 10% citric acid solution, and extracted with EtOAc. The organic layer was dried and evaporated to afford a pale yellow solid (520 mg): MP 260°C (dec); IR (KBr) 1760, 1680, 1610 cm^{-1}.

The above pale yellow solid was dissolved in a mixture of TFA (10 ml) and anisole (1.5 ml) with ice-cooling. After stirring for 1 hour, the mixture was concd. Et_2O was added to the residue and the mixture was stirred for 15 minutes with ice-cooling. The separated solids were collected by suction and dissolved in H_2O, and then adjusted to pH 7 by the addition of 5% $NaHCO_3$ solution. The aqueous solution was chromatographed on a Diaion HP-20 column. Elution with 5% aq THF followed by lyophilization yielded the desired compound as a powder (228 mg, 16.4%): MP 240~250°C(dec); IR (KBr) 1765, 1650 cm^{-1}; NMR (DMSO-d_6) δ 3.2~4.0 (2H, br s, C2-CH_2), 3.94 (3H, s, tetrazole-CH_3), 4.3 (2H, s, C3-CH_2), 5.04 (1H, br d, C6-H), 5.40 (1H, br s, thiazole-CHCO), 5.60 and 5.65 (1H, m, C7-H), 6.42 and 6.57 (1H, 2×s, thiazole 5-H), 7.08 (2H, s, NH_2), 8.52 (1H, br s, CONH), 9.20 (1H, br s, CONH).

Anal Calcd for $C_{17}H_{17}N_9O_7S_3$: C 36.75, H 3.08, N 22.69.
Found: C 36.87, H 3.22, N 22.38.

7β-[DL-2-(Malonamido)-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**6**)

1) In a manner similar to that described for the preparation of mono *tert*-butyl oxalate, mono *tert*-butyl malonate was prepared from *tert*-butyl ethyl malonate.[8] IR (KBr) 1730 cm^{-1}; NMR ($CDCl_3$) δ 3.33 (2H, s, CH_2), 1.45 (9H, s, *tert*-Bu).

2) Compound **6** was prepared in a manner similar to the preparation of **5** using mono *tert*-butyl malonate instead of mono *tert*-butyl oxalate. MP 200~230°C (dec); IR (KBr) 1765, 1700 cm^{-1}; NMR (DMSO-d_6) δ 3.30 (2H, s, CH_2CO), 3.60 (2H, br s, C2-CH_2), 3.92 (3H, s, tetrazole-CH_3), 4.62 (2H, br s, C3-CH_2), 4.99 (1H, br d, C6-H), 6.38 and 6.51 (1H, 2×s, thiazole 5-H).

Anal Calcd for $C_{18}H_{19}N_9O_7S_3$: C 37.95, H 3.36, N 22.13.
Found: C 37.99, H 3.36, N 22.09.

General Procedure for the Preparation of **7**, **8** and **10**: A Typical Procedure is Described for the Preparation of 7β-[DL-2-Amidinoacetamido-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-terazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**7**)

To a suspension of amidinoacetic acid hydrochloride (1.1 g, 8 mmol) in dried CH_2Cl_2 (60 ml) was added phosphorus pentachloride (3.33 g, 16 mmol), and the mixture was stirred for 5 hours at room

temp. The precipitates formed were collected by filtration and washed with CH_2Cl_2 to afford acid chloride hydrochloride (740 mg).

The above acid chloride (236 mg, 1.5 mmol) was added with stirring to a cold solution of silylated compound **II**, prepared by adding *N,O*-bis(trimethylsilyl)acetamide (1.2 g, 3 mmol) to a suspension of **II** (484 mg, 1 mmol) in CH_3CN (8 ml). Propylene oxide (0.45 ml, 6.6 mmol) was added to the mixture, and further stirring was performed with ice-cooling for 1 hour. The solution was then allowed to warm slowly to 5°C and stirred for another hour. The reaction mixture was poured into 50% THF (16 ml), concd to remove solvent, diluted with H_2O, and washed with EtOAc. The aqueous layer was made to pH 6 with 5% $NaHCO_3$ and subjected to column chromatography (Diaion HP-20). The column was washed with H_2O and eluted with 15% aq MeOH. The eluate containing the desired compound was evaporated and then lyophilized to give the title compound (165 mg, 29.1%): MP 180~185°C (dec); IR (KBr) 1760, 1690, 1610 cm^{-1}; NMR (D_2O+pyridine-d_5) δ 3.3~4.0 (2H, br s, C2-CH_2), 3.91 (1H, s, CH_2CO), 4.06 and 4.08 (3H, 2×s, tetrazole-CH_3), 4.35 (2H, br s, C3-CH_2), 5.73 (1H, s, thiazole-CHCO), 5.83 and 5.93 (1H, 2×d, J=5 Hz, C7-H), 6.85 and 6.88 (1H, 2×s, thiazole 5-H).

Anal Calcd for $C_{18}H_{21}N_{11}O_5S_3 \cdot H_2O$: C 36.92, H 3.96, N 26.31.
Found: C 36.59, H 3.82, N 26.01.

In a similar manner, compound **8** was prepared from formiminoglycine.[9] MP 178~195°C (dec); IR (KBr) 1760, 1715, 1660, 1590 cm^{-1}; NMR (D_2O+pyridine-d_5) δ 3.4~3.8 (2H, m, C2-CH_2), 4.04 and 4.06 (3H, 2×s, tetrazole-CH_3), 4.2~4.5 (2H, br s, C3-CH_2), 4.41 and 4.43 (1H, 2×s, CH_2CO), 5.17 and 5.21 (1H, 2×d, J=5 Hz, C6-H), 5.64 (1H, s, thiazole-CHCO), 5.73 and 5.84 (1H, 2×d, J=5 Hz, C7-H), 6.82 and 6.85 (1H, 2×s, thiazole 5-H), 8.04 and 8.13 (1H, 2×s, N=CH-N).

Anal Calcd for $C_{18}H_{21}N_{11}O_5S_3 \cdot H_2O$: C 36.92, H 3.96, N 26.31.
Found: C 37.19, H 3.83, N 26.54.

Again in a similar manner, compound **10** was prepared from guanidinoacetic acid.[8] MP 180~190°C (dec); IR (KBr) 1765, 1670, 1520 cm^{-1}; NMR (D_2O+pyridine-d_5) δ 3.3~4.2 (2H, br s, C2-CH_2), 4.07 and 4.09 (3H, 2×s, tetrazole-CH_3), 4.33 (4H, br s, C3-CH_2 and CH_2CO), 5.75 (1H, s, thiazole-CHCO), 5.84 and 5.94 (1H, 2×d, J=5 Hz, C7-H), 6.87 and 6.90 (1H, 2×s, thiazole 5-H).

Anal Calcd for $C_{18}H_{22}N_{12}O_5S_3 \cdot H_2O$: C 35.99, H 4.03, N 27.98.
Found: C 35.70, H 3.87, N 27.67.

7β-[DL-2-(Guanidinocarbonylamino)-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**9**)

To an ice-cooled solution of 4-guanidinosemicarbazide dihydrochloride[10] (570 mg, 3 mmol) in H_2O (30 ml) was added sodium nitrite (207 mg, 3 mmol) and the mixture was stirred for 30 minutes at the same temp. The solution was adjusted to pH 7 with triethylamine (303 mg, 3 mmol). To this solution was added a solution of compound **II** (970 mg, 2 mmol) and triethylamine (202 mg, 2 mmol) in 50% aq THF (60 ml). With ice-cooling, stirring was continued for 3 hours. The solution was evaporated to remove THF, and the residue was adjusted to pH 4 with 10% citric acid. The pale yellow powder formed was collected by filtration and subjected to column chromatography (Diaion HP-20). The column was washed with H_2O and eluted with 20% MeOH. The eluates containing the product were combined, evaporated to remove MeOH and lyophilized to afford the title compound (650 mg, 38.1%): MP 204~207°C (dec); IR (KBr) 1760, 1680, 1600 cm^{-1}; NMR (DMSO-d_6) δ 3.3~4.0 (2H, br s, C2-CH_2), 3.95 (3H, s, tetrazole-CH_3), 4.35 (2H, br s, C3-CH_2), 5.05 (1H, br s, C6-H), 5.38 (1H, m, thiazole-CHCO), 5.59 (1H, m, C7-H), 6.53 (1H, s, thiazole 5-H).

Anal Calcd for $C_{17}H_{20}N_{12}O_5S_3 \cdot H_2O$: C 34.81, H 3.78, N 28.65.
Found: C 34.70, H 4.26, N 28.39.

Acknowledgment

The authors wish to thank Dr. Y. OSADA and the staff of the Laboratory of Microbiology, and the Analytical Section of the Research Institute of Daiichi Seiyaku Co., Ltd.

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