A NEW CEPHALOSPORIN. SCE-963: 7-[2-(2-AMINOTHIAZOL-4-YL)-ACETAMIDO]-3-[[[1-(2-DIMETHYLAMINOETHYL)-1H-TETRAZOL-5-YL]-THIO]METHYL]CEPH-3-EM-4-CARBOXYLIC ACID

CHEMISTRY AND STRUCTURE-ACTIVITY RELATIONSHIPS[1]

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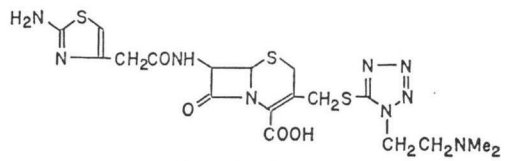
The synthesis and the *in vitro* and *in vivo* antimicrobial activities of a series of 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins (**1**) having varied 3-substituents, such as methyl, hydroxymethyl, acetoxymethyl, pyridiniomethyl and heterocyclicthiomethyls, are described. The derivatives having five membered heterocyclicthiomethyls exhibited strong inhibitory activities against Gram-negative organisms including some strains of *Escherichia coli* and *Proteus morganii* which are insensitive to cefazolin and cephaloridine. Pronounced activities were noted with 7-[2-(2-aminothiazol-4-yl)-acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid (**1y**; SCE-963).

Synthesis of cephalosporins[2] currently in clinical use and of those in their research and development stages involves introduction of acyl groups onto the amino moiety of the 7-aminocephalosporins. These 7-acyl groups together with the substituents on the methylene at the 3-position confer varied antimicrobial spectra as well as pharmacokinetic properties on the cephalosporins. Thus, a number of carboxylic acids have been synthesized, converted into activated forms, like mixed anhydrides or acid halides, and then condensed with 7-aminocephalosporins to obtain a wide variety of cephalosporins.

We have developed a novel method to construct 2-(2-aminothiazol-4-yl)acetamido (**1**) and 2-(2-oxo-4-thiazolin-4-yl)acetamido (**5**) cephalosporins by first preparing the 4-halogeno-3-oxobutyrylamino derivatives (**3**)[3] and then cyclizing as summarized in Scheme 1.

Among the cephalosporins synthesized by this route, the title compound with the code number SCE-963 (**1y**), which exhibited pronounced antimicrobial activity together with excellent pharmacokinetic properties, is now under clinical trial.

This paper deals with the synthesis and the structure-activity relationships of SCE-963 and the related compounds.



1y : SCE-963

Synthesis

The cephalosporins in the present paper except for **1d** and **1e** were prepared by one of the following three processes (A, B, C) outlined in Scheme 1.

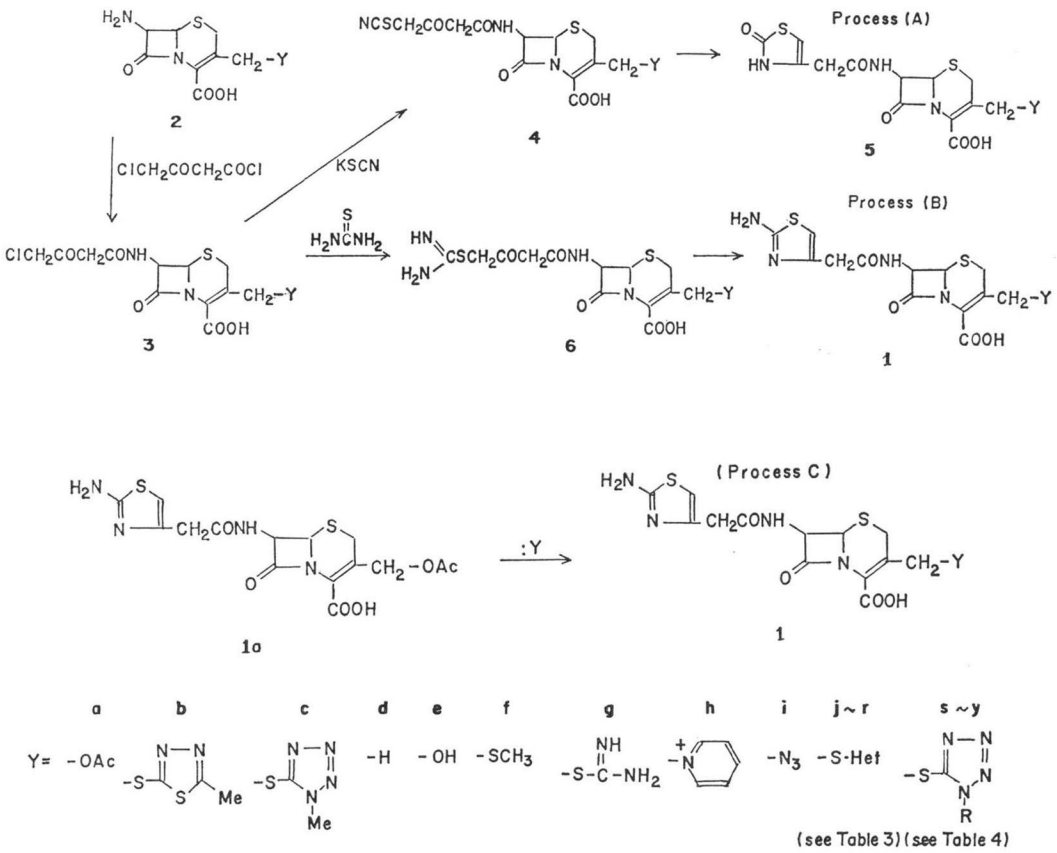
Process (A): Acylation of 7-aminoceph-3-em-4-carboxylic acids (**2**) with 4-chloro-3-oxobutyryl

chloride gave 7-(4-chloro-3-oxobutyrylamino)cephalosporins (**3**). Treatment of **3** with potassium thiocyanate in acetonitrile afforded 7-(3-oxo-4-thiocyanatobutyrylamino)cephalosporins (4). Cyclization of **4** in pH 6.4 phosphate buffer gave 7-[2-(2-oxo-4-thiazolin-4-yl)acetamido]cephalosporins (**5**).

Process (B): Treatment of **3** with thiourea in the presence of a base such as sodium bicarbonate or di-*n*-butylamine gave 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins (**1**) *via* intermediates **6**.

Process (C): Nucleophilic displacement of the acetoxy group of 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporanic acid (**1a**) with an appropriate nucleophile such as methanethiol, thiourea, pyridine, sodium azide or a heterocyclic thiol by conventional methods[4] gave **1**.

Scheme 1.

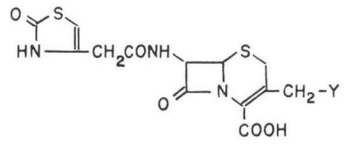


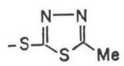
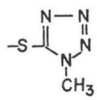
The 3-methyl compound (**1d**) was prepared from 7-aminodesacetoxycephalosporanic acid 2-methylsulfonylethyl ester[5] by the same reactions given in the process (B) followed by the ester hydrolysis.

3-Hydroxymethyl compound (**1e**) was prepared from **1a** by enzymic hydrolysis with a lipase produced by *Rhizopus* sp. NR 400.

Tetrazole thiols used for the preparation of **1s**~**y** were prepared by modifications of the known methods[6,7]: (1) the reaction of (substituted alkyl)isothiocyanates and sodium azide; (2) the reaction of methyl N-(substituted alkyl)dithiocarbamates and sodium azide; or (3) modification of the functional groups in various 1-(substituted alkyl)tetrazole thiols.

Table 1. *In vitro* activity of 7-[2-(2-oxo-4-thiazolin-4-yl)acetamido]cephalosporins

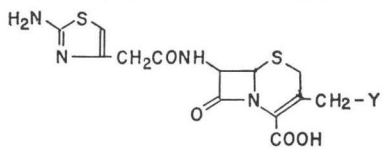


Compound	Y	Process	Minimum inhibitory concentration (mcg/ml)[a]								Geometric mean[b]
			S.a.(S)	*S.a.* (R)	*E.c.* (S-1)	*E.c.* (S-2)	*E.c.* (R)	*K.p.*	*P.v.*	*P.m.*	
5a	-OAc	A	0.78	1.56	6.25	12.5	>100	6.25	25	100	10.51
5b		A	0.20	0.78	1.56	0.78	>100	1.56	6.25	50	3.13
5c		A	≤0.20	0.78	0.78	0.39	50	1.56	1.56	50	2.03
cefazolin			0.39	0.78	1.56	1.56	100	1.56	6.25	100	4.05
cephaloridine			0.05	0.39	3.13	1.56	>100	1.56	6.25	>100	3.72

a: The MIC's were determined by the two-fold serial dilution method on Tripticase soy agar (BBL). Organisms are: *S.a.* (S), *Staphylococcus aureus* 209P; *S.a.* (R), *Staphylococcus aureus* 1840 (penicillin G resistant); *E.c.* (S-1), *Escherichia coli* NIHJ JC-2; *E.c.* (S-2), *Escherichia coli* O-111; *E.c.* (R), *Escherichia coli* T-7 (cefazolin and cephaloridine resistant); *K. p.*, *Klebsiella pneumoniae* DT; *P.v.*, *Proteus vulgaris* IFO-3988; *P.m.*, *Proteus morganii* IFO-3168 (cefazolin and cephaloridine resistant).

b: The MIC data being less than 0.2 or more than 100 were taken into the calculation as 0.2 or 100 respectively.

Table 2. *In vitro* activity of 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins

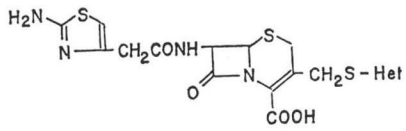


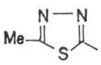
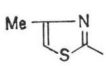
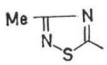
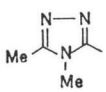
Compound	Y	Process	Minimum inhibitory concentration (mcg/ml)[a]								Geometric mean[b]
			S.a (S)	*S.a.* (R)	*E.c.* (S-1)	*E.c.* (S-2)	*E.c.* (R)	*K.p.*	*P.v.*	*P.m.*	
1d	-H	B	12.5	12.5	25	6.25	50	6.25	25	>100	19.28
1e	-OH	c	3.13	3.13	50	6.25	>100	12.5	12.5	>100	16.21
1f	$-SCH_3$	C	0.39	1.56	25	6.25	>100	1.56	1.56	100	6.25
1g		C	0.39	1.56	12.5	6.25	50	3.13	3.13	>100	6.25
1h		C	≤0.2	0.78	1.56	0.78	100	1.56	1.56	>100	2.87
1i	$-N_3$	C	0.39	0.78	1.56	0.78	100	≤0.2	0.78	100	2.21
1a	-OAc	B	0.78	1.56	1.56	0.39	50	0.39	0.39	50	2.03

a, b: See footnotes a, b in Table 1.

c : Hydrolysis of **1a** by an enzyme.

Table 3. *In vitro* activity of 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins



Compound	-Het.	Process	Minimum inhibitory concentration (mcg/ml)[a]								Geometric mean[b]
			S.a. (S)	*S.a.* (R)	*E.c.* (S-1)	*E.c.* (S-2)	*E.c.* (R)	*K.p.*	*P.v.*	*P.m.*	
1j		C	0.2	0.78	6.25	1.56	50	0.78	0.78	25	2.41
1k		C	≤0.2	0.78	6.25	1.56	12.5	0.78	0.78	50	2.21
1l		C	0.39	0.78	6.25	0.78	25	0.39	0.39	25	1.86
1m		C	0.39	1.56	0.78	0.39	6.25	0.39	0.39	50	1.31
1b		C	0.39	0.78	1.56	0.39	25	0.39	0.78	3.13	1.20
1n		C	0.78	1.56	0.2	<0.05	25	0.1	0.39	25	1.10
1o		C	0.39	0.78	0.78	0.78	6.25	0.2	0.39	25	1.10
1p		C	≤0.2	0.78	1.56	0.78	12.5	0.39	0.39	6.25	1.10
1q		C	≤0.2	≤0.2	3.13	0.39	50	0.39	0.2	1.56	0.85
1r		C	0.39	1.56	0.2	0.1	25	0.1	0.2	1.56	0.66
1c		B	0.39	0.78	0.39	0.2	6.25	<0.1	0.39	3.13	0.66

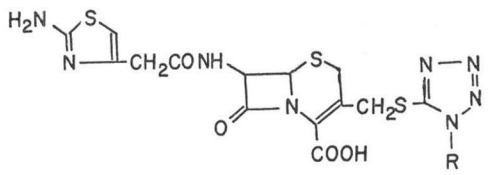
a, b: See footnotesa, b in Table 1.

The cephalosporins thus prepared were characterized and tested as free acids or as their sodium salts (Table 6).

Structure-Activity Relationships

The minimum inhibitory concentration (MIC) values of the new series of cephalosporins against a variety of Gram-positive and Gram-negative bacteria were determined by the two-fold serial agar dilu-

Table 4. *In vitro* activity of 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins



Compound	R	Process	Minimum inhibitory concentration (mcg/ml)[a]								Geometric mean[b]
			S.a. (S)	*S.a.* (R)	*E.c.* (S-1)	*E.c.* (S-2)	*E.c.* (R)	*K.p.*	*P.v.*	*P.m.*	
1s	$-CH_2CONMe_2$	C	1.56	1.56	1.56	≤0.2	25	≤0.2	≤0.2	25	1.43
1t	$-CH_2CH_2NEt_2$	C	0.78	1.56	0.39	0.10	12.5	0.2	1.56	12.5	1.20
1u	$-CH_2CH_2OH$	C	1.56	1.56	0.78	≤0.2	12.5	≤0.2	≤0.2	3.13	0.93
1v	$-(CH_2)_3NMe_2$	C	0.39	0.78	0.39	0.2	6.25	0.39	0.39	3.13	0.72
1w	$-CH_2CH_2NHMe$	C	0.39	0.78	0.39	≤0.2	3.13	≤0.2	0.39	6.25	0.66
1c	$-Me$	B	0.39	0.78	0.39	0.2	6.25	<0.1	0.39	3.13	0.66
1x	$-CH_2CONH_2$	C	0.78	1.56	0.39	≤0.2	3.13	≤0.2	≤0.2	≤0.2	0.46
1y (SCE-963)	$-CH_2CH_2NMe_2$	C	0.39	1.56	0.2	≤0.2	1.56	≤0.2	0.78	≤0.2	0.43

a, b: See footnotesa, b in Table 1.

tion method. The comparative results with cefazolin (CEZ) and cephaloridine (CER) are summarized in Tables 1, 2, 3 and 4. For the convenience of comparison, the compounds in each table are arranged in the increasing order of activity. The order was judged by the geometric mean of MIC's of each compound against 8 organisms.

Table 1 lists a series of 7-[2-(2-oxo-4-thiazolin-4-yl)acetamido]cephalosporins (**5**). Improvement in the activity against Gram-negative bacteria was observed with the variations of 3-substituents in the order of acetoxymethyl (**5a**), methylthiadiazolethiomethyl (**5b**) and methyltetrazolethiomethyl (**5c**). This order is the same as that reported for other series of cephalosporins.[8]

The activity of a series of 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins (**1**) with commonly used 3-substituents is shown in Table 2. Replacement of the acetoxy group of **1a** with hydrogen, hydroxy, methylthio, carbamimidoylthio, pyridinio and azido groups resulted in a decrease in the activity against Gram-negative bacteria.

The activities of the compounds (**1b, c, j**~**r**) wherein the acetoxy group of **1a** is replaced by heterocyclic thiols are shown in Table 3. Replacement by five membered heterocyclic thiols generally enhanced activities against both Gram-positive and Gram-negative bacteria. Displacement by six-membered heterocyclic thiols, however, did not increase the activity against Gram-negative bacteria.

When comparisons were made between the compounds in the 2-oxo-4-thiazoline series (**5**) and those in the 2-aminothiazol series (**1**), *i.e.* **5a** *vs.* **1a**, **5b** *vs.* **1b** and **5c** *vs.* **1c**, the former compounds were less active against Gram-negative bacteria than the latter compounds.

Of both series, the 3-methyltetrazolethiomethyl compound (**1c**) was the most active. Against most of the Gram-negative organisms tested, **1c** was 4~32 times as active as CEZ and CER.

Since **1c** was sparingly soluble in an aqueous solution at physiological pH, replacement of the methyl group attached to the tetrazole ring of **1c** with an alkyl group carrying a hydrophilic function was then attempted.

Table 5. Protective activity of 7-[2-(2-aminothiazol-4-yl)acetamido]cephalosporins in mice infected with *E. coli* O-111

Compound	1a	1c	1r	1t	1y (SCE-963)	CEZ
ED_{50} (mg/kg)*	1.79	0.112	0.173	0.155	0.086	1.55

* The ED_{50} values are expressed as the dose of compound which afforded protection to 50% of the mice (male mice; Slc-ICR strain) challenged intraperitoneally with 10^5 CFU/animal of test organism. A single dose (5 mice per one dose) was administered subcutaneously immediately after challenge.

Table 6. Empirical formulas for cephalosporins reported

Compound	Formula[a]	Compound	Formula[a]
3a	$C_{14}H_{15}ClN_2O_7S$	**1i**	$C_{13}H_{12}N_7O_4S_2Na \cdot 1.5H_2O$[l]
b	$C_{15}H_{15}ClN_4O_5S_3 \cdot 1/3Et_2O \cdot$ 0.5AcOEt[b]	**j**	d
		k	d
c	$C_{14}H_{15}ClN_6O_5S_2 \cdot 2/3Et_2O$[c]	**l**	$C_{15}H_{14}N_7O_4S_3Na \cdot 2H_2O$[m]
4a	$C_{15}H_{15}N_3O_7S_2 \cdot 0.5Et_2O$	**m**	$C_{17}H_{17}N_6O_4S_3Na \cdot 2H_2O$
b	d	**n**	$C_{16}H_{16}N_7O_4S_3Na \cdot 2.5H_2O$
c	$C_{15}H_{15}N_7O_5S_3 \cdot 0.2Et_2O \cdot$ 0.2AcOEt[e]	**o**	$C_{15}H_{14}N_7O_4S_3Na \cdot 1.5H_2O$[n]
		p	$C_{17}H_{17}N_5O_4S_4 \cdot 1.5H_2O$
5a	$C_{15}H_{15}N_3O_7S_2$	**q**	$C_{16}H_{16}N_6O_4S_4 \cdot H_2O$[o]
b	$C_{16}H_{15}N_5O_5S_4 \cdot AcOEt \cdot H_2O$[f]	**r**	$C_{17}H_{18}N_7O_4S_3Na \cdot 3H_2O$
c	$C_{15}H_{15}N_7O_5S_3 \cdot AcOEt$[g]	**s**	$C_{18}H_{20}N_9O_5S_3Na \cdot 2H_2O$
1a	$C_{15}H_{16}N_4O_6S_2 \cdot 1.5H_2O$[h]	**t**	$0.3(C_{20}H_{26}N_9O_4S_3Na) \cdot 0.7(C_{20}H_{27}N_9O_4S_3) \cdot 1.5H_2O$
b	$C_{16}H_{15}N_6O_4S_4Na \cdot 2.5H_2O$[i]		
c	$C_{15}H_{15}N_8O_4S_3Na \cdot 2.5H_2O$[j]	**u**	$C_{16}H_{17}N_8O_5S_3Na \cdot 1.5H_2O$
d	$C_{13}H_{13}N_4O_4S_2Na \cdot 2H_2O$[k]	**v**	$C_{19}H_{24}N_9O_4S_3Na \cdot 0.5H_2O$[p]
e	$C_{13}H_{13}N_4O_5S_2Na \cdot H_2O$	**w**	$C_{17}H_{21}N_9O_4S_3 \cdot HCl \cdot 1.5H_2O$[q]
f	$C_{14}H_{15}N_4O_4S_3Na \cdot H_2O$	**x**	$C_{16}H_{16}N_9O_5S_3Na \cdot 3H_2O$[r]
g	d	**y**	$C_{18}H_{23}N_9O_4S_3 \cdot 1.5H_2O$
h	$C_{18}H_{17}N_5O_4S_2 \cdot 2.5H_2O$		

a: Unless otherwise indicated, analytical results for C, H and N for all compounds were within 0.4% of the theoretical value.
b: N, calcd., 10.54; found, 10.09.
c: C, calcd., 40.33; found, 39.16. N, calcd., 16.93; found, 15.77.
d: Not determined.
e: N, calcd., 19.53; found, 18.59.
f: H, calcd., 4.26; found, 3.80.
g: C, calcd., 40.92; found, 40.51. N, calcd., 17.57; found, 16.61.
h: H, calcd., 4.36; found, 3.75.
i: N, calcd., 15.23; found, 14.82.
j: H, calcd., 3.76; found, 3.33. N, calcd., 20.92; found, 19.86.
k: C, calcd., 37.86; found, 38.69. N, calcd., 13.59; found, 12.77.
l: N, calcd., 22.52; found, 21.45.
m: N, calcd., 19.17; found, 17.83.
n: N, calcd., 19.51; found, 18.20.
o: N, calcd., 15.92; found, 16.35.
p: H, calcd., 4.42; found, 5.02. N, calcd., 22.09; found, 21.51.
q: H, calcd., 4.38; found, 3.91.
r: H, calcd., 3.77; found, 3.03. N, calcd., 21.45; found, 19.02.

As shown by the data in Table 4, although the variations of the side chain of the tetrazole did not cause significant change in overall activity, five out of seven compounds in Table 4 were slightly less active than the parent **1c**. However two compounds, **1x** and **1y** (SCE-963), which carry a carbamoylmethyl and a dimethylaminoethyl, respectively, instead of the methyl of **1c**, exhibited improved activity against Gram-negative bacteria especially against β-lactamase producing strains, namely *Escherichia coli* T-7 and *Proteus morganii* IFO-3168. Against these two organisms they were more than 32 times as active as CEZ and CER. Of the two compounds, **1y** was selected for further study from the standpoint of better solubility in an aqueous solution around physiological pH and easier synthetic approach.

In vivo studies of some compounds were carried out in mice infected with *E. coli* O-111 (Table 5). Table 5 shows that their order in protective effect (ED_{50} values) nearly paralleled that of the *in vitro* activity. Among them, **1y** (SCE-963) was the most active and 18 times as potent as CEZ in the protection test.

As a result of the present preliminary studies SCE-963 was selected for further evaluation.

Experimental

Infrared (IR) spectra were measured in KBr disks using a Hitachi EPI-S_2 spectrophotometer. NMR spectra were recorded on a Varian HA-100 spectrometer using tetramethylsilane as a standard. All melting points are uncorrected.

Process (A)

7-(4-Chloro-3-oxobutyrylamino)cephalosporins (**3a, b, c**)

To a cold (−50 ~ −30°C) stirred solution of diketene (10.9 g, 0.13 mol) in CH_2Cl_2 (50 ml) was added a solution of Cl_2 (9.2 g, 0.13 mol) in CCl_4 (82 ml) and the stirring was continued for 1 hour. The solution was added rapidly to a cold (−20°C) stirred solution of 7-aminoceph-3-em-4-carboxylic acid (**2a, b** or **c**) (0.1 mol) and Et_3N (20.2 g, 0.2 mol, in cases of **2a** and **2b**) or di-*n*-butylamine (26 g, 0.2 mol, in case of **2c**) in CH_2Cl_2 (200 ml). The solution was warmed to room temperature during 1.5 hours and shaken with AcOEt (1.5 liters) and 10% H_3PO_4 (1 liter). The organic layer was washed with water, dried and evaporated. The residue was triturated with Et_2O, filtered and dried to give the cephalosporins (**3a, b, c**).

3a: Reported in the previous paper.[3]

3b: IR 1775, 1720, 1530, 1370, 1240, 1060 cm^{-1}; NMR (DMSO-d_6) δ 2.67 (s, thiadiazole-CH_3), 3.50 & 3.78 (ABq, J=18 Hz, C_2–H_2), 3.57 (s, $COCH_2CO$), 4.20 & 4.50 (ABq, J=12 Hz, C_3–CH_2), 4.53 (s, $ClCH_2$), 5.07 (d, J=4.5 Hz, C_6–H), 5.65 (dd, J=4.5 & 8 Hz, C_7–H), 9.05 (d, J=8 Hz, CONH).

3c: IR 1780, 1720, 1540, 1390, 1240, 1170, 700 cm^{-1}; NMR (DMSO-d_6) δ 3.57 & 3.79 (ABq, J=18 Hz, C_2–H_2), 3.56 (s, $COCH_2CO$), 3.91 (s, tetrazole-CH_3), 4.20 & 4.37 (ABq, J=13 Hz, C_3–CH_2), 4.52 (s, $ClCH_2$), 5.07 (d, J=5 Hz, C_6–H), 5.67 (dd, J=5 & 8 Hz, C_7–H), 9.05 (d, J=8 Hz, CONH).

7-(3-Oxo-4-thiocyanatobutyrylamino)cephalosporins (**4a, b, c**)

A mixture of **3** (1 mmol) and KSCN (0.15 g, 1.5 mmol) in CH_3CN (10 ml) was stirred at room temperature for 16 hours. The solution was evaporated *in vacuo* and the residue was mixed with saturated NaCl (10 ml). The mixture was acidified with 50% H_3PO_4 and extracted with AcOEt. The extract was washed with saturated NaCl, dried and evaporated *in vacuo*. Trituration of the residue with Et_2O afforded the cephalosporins (**4a, b, c**).

4a: IR 2160 (CN), 1785, 1730 cm^{-1}; NMR (DMSO-d_6) δ 2.01 (s, $OCOCH_3$), 3.42 & 3.66 (ABq, J=18 Hz, C_2–H_2), 3.62 (s, $COCH_2CO$), 4.37 (s, SCH_2CO), 4.68 & 5.00 (ABq, J=12 Hz, C_3–CH_2), 5.09 (d, J=4.5 Hz, C_6–H), 5.67 (dd, J=4.5 & 8 Hz, C_7–H), 9.06 (d, J=8 Hz, CONH).

4b: Immediately used for the following reaction.

4c: IR 2160 (CN), 1780, 1720, 1540, 1365, 1240, 1170 cm^{-1}; NMR (DMSO-d_6) δ 3.52 & 3.83 (ABq, J=18 Hz, C_2–H_2), 3.66 (s, $COCH_2CO$), 3.98 (s, tetrazole-CH_3), 4.24 & 4.40 (ABq, J=13 Hz, C_3–CH_2), 4.42 (s, SCH_2CO), 5.10 (d, J=4.5 Hz, C_6–H), 5.70 (dd, J=4.5 & 8 Hz, C_7–H), 9.09 (d, J=8 Hz, CONH).

7-[2-(2-Oxo-4-thiazolin-4-yl)acetamido]cephalosporins (**5a**, **b**, **c**)

A solution of **4** (1 mmol) and $NaHCO_3$ (84 mg, 1 mmol) in phosphate buffer of pH 6.4 (5 ml) was allowed to stand at room temperature overnight. The solution was acidified to pH 3 with 50% H_3PO_4, saturated with NaCl and extracted with AcOEt. The extract was washed with saturated NaCl, dried and evaporated. The residue was triturated with Et_2O to afford the cephalosporins (**5a**, **b**, **c**).

5a: IR 1775, 1715, 1665, 1530, 1380, 1240 cm^{-1}; NMR (DMSO-d_6) δ 1.99 (s, $OCOCH_3$), 3.32 (s, CH_2CO), 3.41 & 3.65 (ABq, J=18 Hz, C_2–H_2), 4.68 & 4.99 (ABq, J=12 Hz, C_3–CH_2), 5.07 (d, J=4.5 Hz, C_6–H), 5.68 (dd, J=4.5 & 8 Hz, C_7–H), 6.00 (s, thiazoline 5–H), 8.94 (d, J=8 Hz, CONH), 11.07 (s, thiazoline N–H).

5b: IR 1786, 1655, 1535 cm^{-1}; NMR (DMSO-d_6) δ 2.63 (s, thiadiazole-CH_3), 3.30 (s, CH_2CO), 3.53 & 3.77 (ABq, J=18 Hz, C_2–H_2), 4.18 & 4.49 (ABq, J=14 Hz, C_3–CH_2), 5.04 (d, J=5 Hz, C_6–H), 5.65 (dd, J=5 & 8 Hz, C_7–H), 5.98 (s, thiazoline 5-H), 8.94 (d, J=8 Hz, CONH).

5c: IR 1785, 1730, 1660 cm^{-1}; NMR (DMSO-d_6) δ 3.34 (s, CH_2CO), 3.70 (m, C_2–H_2), 3.92 (s, tetrazole-CH_3), 4.30 (m, C_3–CH_2), 5.07 (d, J=5 Hz, C_6–H), 5.68 (dd, J=5 & 8 Hz, C_7–H), 6.01 (s, thiazoline 5–H), 8.98 (d, J=8 Hz, CONH).

Process (B)

7-[2-(2-Aminothiazol-4-yl)acetamido]cephalosporanic acid (**1a**)

To a stirred solution of thiourea (0.602 g) and $NaHCO_3$ (0.664 g) in water (80 ml) and THF (40 ml) was added **3a** (3.805 g). After stirring for 1 hour, the solution was concentrated *in vacuo*. The precipitate was collected, washed and dried to give **1a** (2.703 g, 83%). IR 1776, 1659, 1545, 1240 (br.) cm^{-1}; NMR (DMSO-d_6) δ 2.01 (s, $OCOCH_3$), 3.38 (s, CH_2CO), 3.40 & 3.63 (ABq, J=18 Hz, C_2–H_2), 4.68 & 4.98 (ABq, J=13 Hz, C_3–CH_2), 5.06 (d, J=5 Hz, C_6–H), 5.68 (dd, J=5 & 8 Hz, C_7–H), 6.23 (s, thiazole 5–H), 6.90 (br. s, NH_2), 8.82 (d, J=8 Hz, CONH).

Sodium 7-[2-(2-aminothiazol-4-yl)acetamido]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]ceph-3-em-4-carboxylate (**1c**)

To a stirred solution of **3c** (22.4 g) and di-*n*-butylamine (6.5 g) in CH_2Cl_2 (160 ml) was added thiourea (4 g) in one portion. After stirring for 3 hours at room temperature, the precipitate was collected, washed with CH_2Cl_2 and then water and dried to give free acid of **1c** (24 g, 98%). To an ice-cold suspension of free acid of **1c** (6.5 g) in water (30 ml) was added 0.5 N NaOH (26.4 ml) for 30 minutes. The solution was concentrated to about 30 ml and chromatographed on an Amberlite XAD-2 (Rohm & Haas) column with water as eluent. The fractions collected were lyophilized to give **1c** (6.03 g, 83% from **3c**). IR 1763 cm^{-1}; NMR (D_2O) δ 3.48 & 3.81 (ABq, J=17 Hz, C_2–H_2), 3.63 (s, CH_2CO), 4.06 (s, tetrazole-CH_3), 4.09 & 4.37 (ABq, J=14 Hz, C_3–CH_2), 5.13 (d, J=5 Hz, C_6–H), 5.68 (d, J=5 Hz, C_7–H), 6.52 (s, thiazole 5–H).

Process (C)

7-[2-(2-Aminothiazol-4-yl)acetamido]cephalosporins (**1b**, **f**, **g**, **i**, **j**~**x**)

A phosphate buffer solution (pH 6.4, 40 ml) containing a mixture of **1a** (0.824 g, 2 mmol) plus either 2.2 mmol of an appropriate heterocyclic thiol, 2.2 mmol of CH_3SH, 2 mmol of thiourea or 2 mmol of NaN_3 and $NaHCO_3$ (0.336 g or 0.168 g in case of NaN_3) was heated at 50~65°C for 7~16 hours (termination of the reaction was checked by thin-layer chromatography). The pH was maintained around 6.4 by the addition of 5% $NaHCO_3$ or 3 N HCl. The reaction mixture was concentrated to about 20 ml and chromatographed on an XAD-2 column eluting subsequently with water, 5% EtOH and 10% EtOH. The fractions containing the desired product were collected and lyophilized to give the cephalosporins. In one case (**1g**), a precipitate was formed during heating, which was collected, washed with water and acetone and dried to give the cephalosporin **1g**. The IR and NMR spectra of the cephalosporins were consistent with the structures.

7-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]-ceph-3-em-4-carboxylic acid (**1y**; SCE-963)

A solution of **1a** (0.824 g), 1-(2-dimethylaminoethyl)-1H-tetrazole-5-thiol (0.346 g) and $NaHCO_3$ (0.168 g) in water (8 ml) was heated at 65~66°C for 1.5 hours. The solution was acidified to pH 3 with 1N HCl and filtered. The filtrate was adjusted to pH 5.8 with 1 N NaOH and chromatographed on

an XAD-2 column by a gradient elution from water to 40% MeOH. The fractions containing the desired product were collected and lyophilized to give **1y** (0.151 g, 14%). IR 1767, 1607, 1515, 1386, 1347 cm^{-1}; NMR (D_2O) δ 3.01 (s, $N(CH_3)_2$), 3.47 & 3.78 (ABq, J=18 Hz, C_2–H_2), 3.59 (s, CH_2CO), 3.78 (t, J=6 Hz, CH_2N), 4.11 & 4.28 (ABq, J=13 Hz, C_3–CH_2), 4.82 (t, J=6 Hz, tetrazole-CH_2), 5.09 (d, J=5 Hz, C_6–H), 5.61 (d, J=5 Hz, C_7–H), 6.49 (s, thiazole 5–H); pKa (H_2O) 2.59, 4.63, 7.13.

1-Substituted alkyl-1H-tetrazole-5-thiol

The thiols were prepared by one of the following three methods. The thiols in this section are designated with the numbers of cephalosporins to which they are incorporated.

Method 1: From the corresponding (substituted alkyl)isothiocyanate on heating with NaN_3 in aqueous EtOH by the procedures described by R. STOLLE *et al.*[6]

1t: mp 122~123°C. **1y**: mp 217~219°C.

Method 2: From the corresponding methyl N-(substituted alkyl)dithiocarbamate, which was formed by the reaction of a substituted alkylamine with CS_2 and MeI, on heating with NaN_3 in aqueous EtOH by the procedures described by R. E. ORTH and J. W. JONES.[7]

1s: mp 195~198°C (dec.). **1v**: mp 192~193°C.

Method 3: By conventional transformations from tetrazole thiols carrying an appropriate functional group.

1u: mp 137~139°C, by $LiAlH_4$ reduction of 1-benzyloxycarbonylmethyl-1H-tetrazole-5-thiol which was prepared by method 2.

1x: mp 200°C (dec.), treatment of 1-benzyloxycarbonylmethyl-1H-tetrazole-5-thiol with NH_3-EtOH.

1w: mp 210~215°C, from **1x** by three subsequent reactions, *i. e.*, reduction of **1x** with B_2H_6 in THF, formylation with HCOOH-Ac_2O and reduction with $NaBH_4$ in AcOH.

7-[2-(2-Aminothiazol-4-yl)acetamido]-3-(pyridiniomethyl)ceph-3-em-4-carboxylate (**1h**)

This compound was prepared from **1a** by displacement of the acetoxy group with pyridine using the method described by ARKLEY, *et al.*[9]

Miscellaneous processes

Sodium 7-[2-(2-aminothiazol-4-yl)acetamido]-3-methylceph-3-em-4-carboxylate (**1d**)

(1) To a cooled (−35°C) and stirred solution of diketene (0.437 g) in CH_2Cl_2 (4 ml) was added Br_2 (0.886 g) in CH_2Cl_2 (1 ml). The mixture was added to a cooled (−30°C) and stirred solution of 2-methylsulfonylethyl 7-amino-3-methylceph-3-em-4-carboxylate[5] (1.3 g) and Et_3N (0.404 g) in CH_2Cl_2 (10 ml). The solution was warmed to room temperature for 1 hour and washed with saturated NaCl, dried and evaporated. Trituration of the residue with Et_2O gave 2-methylsulfonylethyl 7-(4-bromo-3-oxobutyrylamino)-3-methylceph-3-em-4-carboxylate (methylsulfonylethyl ester of **3d**, 1.6 g, 84%), mp 58~60°C (dec.), whose IR and NMR spectra were consistent with the structure.

(2) A solution of the methylsulfonylethyl ester of **3d** (3 g) and thiourea (0.5 g) in acetone (15 ml) was stirred at room temperature for a few minutes. The precipitate was collected and dissolved in a solution of $NaHCO_3$ (0.5 g) in water (15 ml). After standing overnight at room temperature, the solution was extracted with AcOEt. The extract was washed with water, dried and concentrated to give the 2-methylsulfonylethyl ester of **1d** as crystals (2.0 g, 74%), mp 143~150°C (dec.), whose IR and NMR spectra were consistent with the structure.

(3) A chilled suspension of 2-methylsulfonylethyl ester of **1d** (0.461 g) in a solution of NaOH (96 mg) in water (10 ml) was stirred for 45 minutes. The solution was saturated with CO_2 and chromatographed on an XAD-2 column with water as eluent. The fractions collected were lyophilized to give **1d** (0.153 g, 33%). IR 1754, 1634, 1514 cm^{-1}; NMR (D_2O) δ 1.97 (s, C_3–CH_3), 3.25 & 3.63 (ABq, J=18 Hz, C_2–H_2), 3.64 (s, CH_2CO), 5.11 (d, J=4.5 Hz, C_6–H), 5.63 (d, J=4.5 Hz, C_7–H), 6.55 (br. s, thiazole 5–H).

Sodium 7-[2-(2-aminothiazol-4-yl)acetamido]-3-hydroxymethylceph-3-em-4-carboxylate (**1e**)

A mixture of **1a** (4.12 g), lipase (trade name Saiken 100, Osaka Bacteriological Institute) (4.12 g), $NaHCO_3$ (0.84 g) and phosphate buffer of pH 7.2 (125 ml) was stirred at 40°C for 8.5 hours, during which the pH was kept at 7.2 by addition of 10% $NaHCO_3$. The mixture was lyophilized to leave the solid

which was extracted with MeOH (400 ml×2). The combined extracts were filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in water (20 ml) and chromatographed on an XAD-2 column with water as eluent. The fractions collected were lyophilized to afford **1e** (2.643 g, 64%). IR 1760, 1655, 1600, 1510, 1400 cm^{-1}; NMR (D_2O) δ 3.44 & 3.68 (ABq, J=18 Hz, C_2–H_2), 3.62 (s, CH_2CO), 4.30 (s, C_3–CH_2), 5.15 (d, J=4.5 Hz, C_6–H), 5.67 (d, J=4.5 Hz, C_7–H), 6.54 (s, thiazole 5–H).

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