STUDIES ON β-LACTAM ANTIBIOTICS

II. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CEPHALOSPORINS WITH SUBSTITUTED 1,3-DITHIETANE DIRECTLY ATTACHED TO THE C-3 POSITION

NORIAKI NAGANO, KOHJI NAKANO, TADAO SHIBANUMA and RYUICHIRO HARA

Central Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd.,
21 Miyukigaoka, Tsukuba City, Ibaraki 305, Japan

(Received for publication October 24, 1990)

Preparation of cephaloosporins bearing a 1,3-dithietane ring attached to the C-3 position using intramolecular rearrangement are described. The diastereoisomers (**6a-I** and **6a-II**) were separated by silica gel column chromatography. These 3-[4-(carbamoyl carboxymethylene)-1,3-dithietan-2-yl]cephems showed comparable activity against Gram-negative bacteria to that of ceftazidime.

We have reported a novel intramolecular rearrangement between isothiazolethioacetamides and 1,3-dithietanecarboxamides at the C-7 position of cephamycin in a previous paper[1].

In a previous paper[2], we described the synthesis and antimicrobial activity of cephalosporins bearing an isothiazolethiomethyl group at the C-3 position.

In this paper, we wish to report the intramolecular rearrangement of cephalosporin from *iso*-thiazolethiomethyl to 1,3-dithietane at the C-3 position. Using this rearrangement, we could synthesize some cephalosporins bearing a 1,3-dithietane ring directly attached to the C-3 position. These compounds (**5a**～**5f**) showed good activities against Gram-negative bacteria.

Chemistry

As we reported earlier[2], 3-[(4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl]-7β-[(*Z*)-2-(2-tritylamino-4-thiazolyl)-2-(*tert*-butoxycarbonylalkoxyimino)acetamido]-3-cephem-4-carboxylic acids (**3a**～**3f**) were synthesized by acylation of 7β-amino-3-(4-carboxy-3-hydroxy-5-isothiazolyl)thiomethyl-3-cephem-4-carboxylic acid (**2**) with (*Z*)-2-(2-tritylamino-4-thiazolyl)-2-(*tert*-butoxycarbonylalkoxyimino)-acetic acids (**1a**～**1f**). Treatment of **3a**～**3f** with triethylamine in a mixture of dioxane and DMSO at room temperature gave the rearranged 3-(1,3-dithietan-4-yl)cephems (**4a**～**4f**). Stepwise deprotec-

Fig. 1. Intramolecular rearrangement of 7-acyl moiety from isothiazolethiomethyl to 1,3-dithietane.

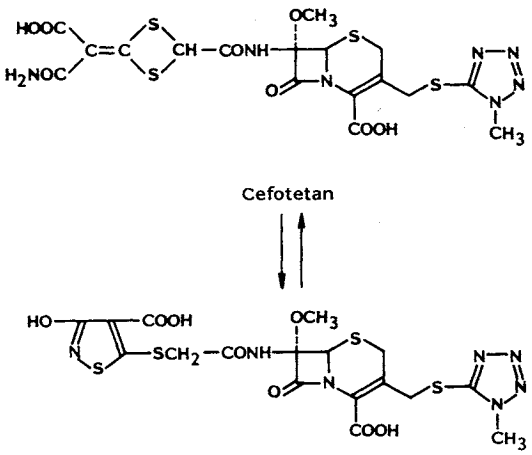
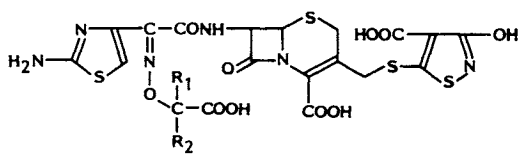
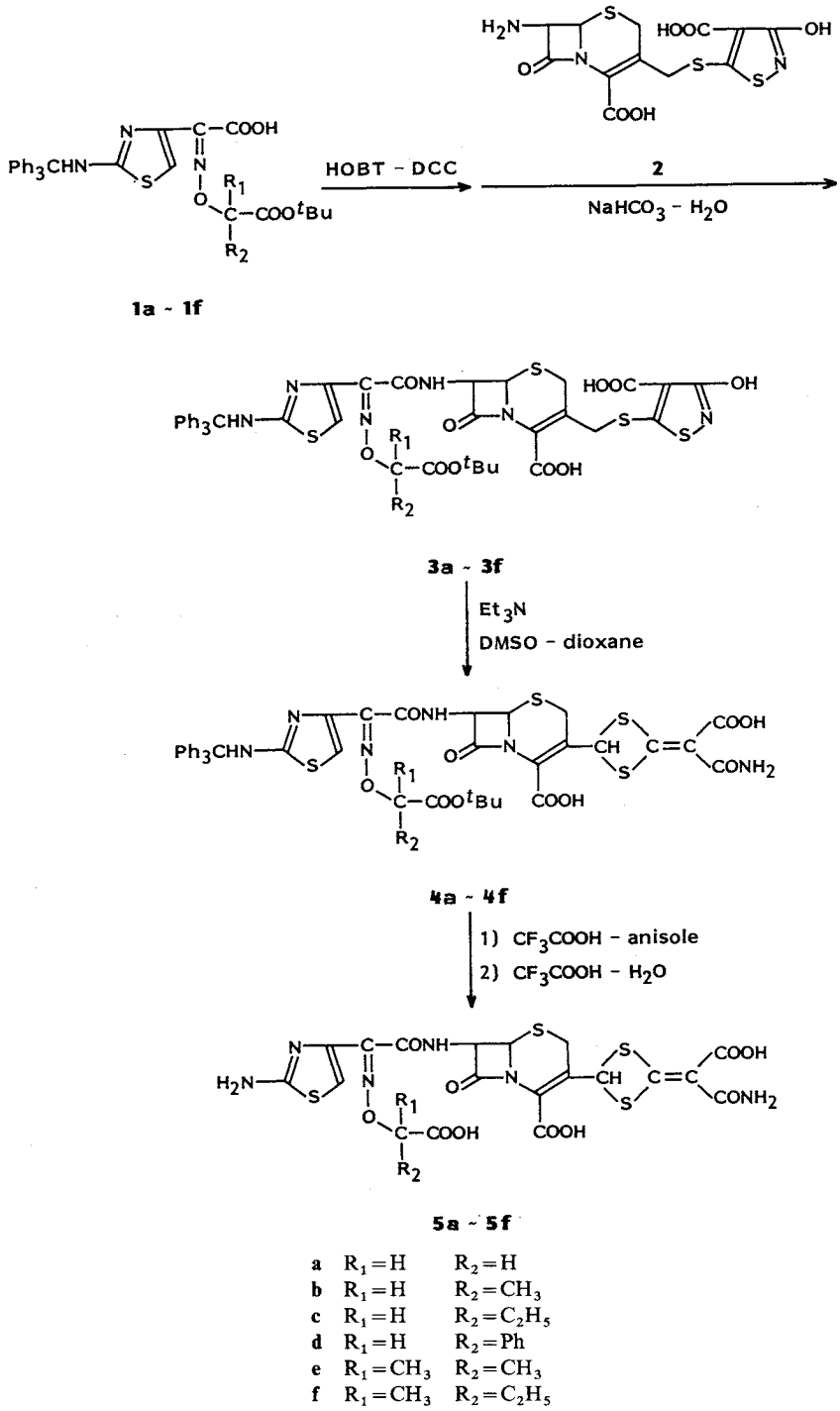


Fig. 2. Structure of YM-13115.



YM-13115 $R_1=CH_3$ $R_2=CH_3$

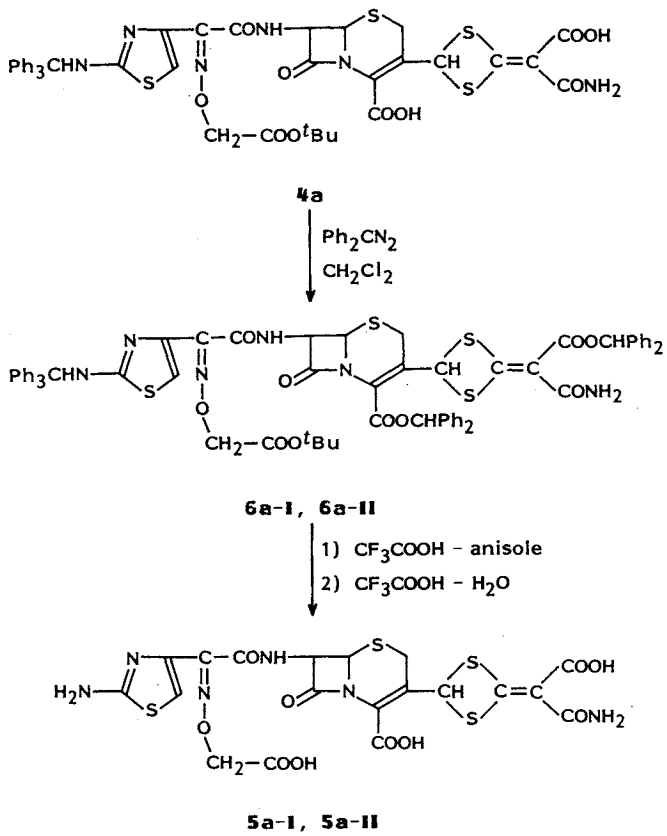
Scheme 1.



a $R_1=H$ $R_2=H$
b $R_1=H$ $R_2=CH_3$
c $R_1=H$ $R_2=C_2H_5$
d $R_1=H$ $R_2=Ph$
e $R_1=CH_3$ $R_2=CH_3$
f $R_1=CH_3$ $R_2=C_2H_5$

tion of the two protective groups of **4a**~**4f** gave the 3-(1,3-dithietan-4-yl)cephems (**5a**~**5f**). The structure of the 3-(1,3-dithietan-4-yl)cephem derivative was confirmed as follows; i) in the NMR spectrum of **4a**, the signal at δ 4.16 (2H, q, C-3-CH_2-) in **3a** was not observed, while a new signal appeared at δ 5.82

Scheme 2.



(1H, s, C-3–CH<), ii) in the IR spectrum of **4a**, new absorption bands at 1680 and 1630 cm^{-1} due to a primary amido group were seen and iii) two diastereoisomers of the cephem triester (**6a**) were readily separated by column chromatography on silica gel (benzene - EtOAc, 3 : 1). The less polar isomer (**6a-I**) (Rf 0.54 (benzene - EtOAc, 3 : 1), $[\alpha]_D^{20}$ −178.5° (*c* 1, CH_2Cl_2)) and the more polar isomer (**6a-II**) (Rf 0.35 (benzene - EtOAc, 3 : 1), $[\alpha]_D^{20}$ −40.4° (*c* 1, CH_2Cl_2)) were obtained. Stepwise removal of the different protecting groups in each isomer produced the free acid (**5a-I**) ($[\alpha]_D^{20}$ −293.5° (*c* 1, DMF)) and (**5a-II**) ($[\alpha]_D^{20}$ −108.5° (*c* 1, DMF)), respectively.

Antibacterial Activity

The *in vitro* antibacterial activities of new 3-[4-(carbamoyl carboxymethylene)-1,3-dithietan-2-yl]cephems (**5a**～**5f**) against selected Gram-positive and Gram-negative organisms are shown in Table 1. In general, these 3-dithietane cephems (**5a**～**5f**) were two to four times less active against all the organisms than the corresponding 3-isothiazolylthiomethyl cephems[2]. However they retained good activity against *Pseudomonas aeruginosa*. As shown in Table 1, the most promising compound was **5f**, the activity of which against four tested strains of *P. aeruginosa* was two times higher than that of ceftazidime (CAZ). There was little difference in the antibacterial activities between **5a-I** and **5a-II**, but the isomer (**5a-I**) was more active than **5a-II** against *Providencia rettgeri* Y-1 and *P. aeruginosa*.

Table 1. Antibacterial activities of 3-[4-(carbamoyl carboxymethylene)-1,3-dithietan-2-yl]cephem compounds (**5a**~**5f**).

Strain	MIC (μg/ml)								
	a	**a-I**	**a-II**	**b**	**c**	**d**	**e**	**f**	CAZ
R_1/R_2:	H/H	H/H	H/H	H/CH_3	H/Et	H/Ph	CH_3/CH_3	CH_3/Et	
Staphylococcus aureus Smith	>100	>100	>100	>100	>100	>100	100	>100	12.5
Escherichia coli O-1	0.78	1.56	1.56	0.78	3.13	3.13	0.39	≦0.2	≦0.2
E. coli Ebara	3.13	1.56	3.13	≦0.2	3.13	1.56	0.39	≦0.2	0.78
Klebsiella pneumoniae ATCC 10031	1.56	0.78	1.56	≦0.2	3.13	0.78	≦0.2	≦0.2	≦0.2
K. pneumoniae V-17	12.5	12.5	50	0.78	6.25	12.5	0.78	0.78	0.39
Serratia marcescens IID 620	0.39	0.39	0.39	12.5	1.56	6.25	1.56	0.78	0.39
Providencia rettgeri Y-1	0.39	0.2	0.39	0.78	1.56	3.13	≦0.2	0.78	3.13
Enterobacter cloacae 963 (MS-1)	3.13	3.13	6.25	6.25	12.5	6.25	—	3.13	0.39
E. aerogenes NY-2	6.25	6.25	12.5	1.56	12.5	6.25	3.13	1.56	6.25
Pseudomonas aeruginosa NCTC 10490	3.13	3.13	3.13	1.56	6.25	3.13	0.78	0.39	0.78
P. aeruginosa IID 5142	6.25	3.13	6.25	1.56	12.5	6.25	0.39	1.56	3.13
P. aeruginosa NC-5	3.13	3.13	6.25	3.13	6.25	6.25	3.13	1.56	3.13
P. aeruginosa 99	12.5	6.25	25	3.13	12.5	12.5	3.13	1.56	3.13

CAZ: Ceftazidime.

Table 2. ^{1}H NMR spectral data of **4**.

Compound			^{1}H NMR δ value (DMSO-d_6) ppm							
No.	R_1	R_2	CONH (1H, d)	Thiazole 5-H (1H, s)	7-H (1H, dd)	3-CH< (1H, d)	6-H (1H, d)	2-H (2H, ABq)	*tert*-Bu (9H, s)	R_1, R_2
4a[a]	H	H	9.45	6.76	5.74	5.72	5.11	3.9	1.42	4.52 (2H, br s)
4b[b]	H	CH_3	9.40	6.72	5.55	5.72	5.14	3.90	1.37	4.50 (1H, m), 1.28 (3H, d)
4c[b]	H	Et	9.39	6.71, 6.72	5.76	5.72	5.12	3.91	1.40	4.34 (1H), 1.76 (2H, m), 0.82 (3H, t)
4d[b]	H	Ph	9.44	6.78, 6.80	5.76	5.74, 5.78	5.12	3.90	1.34	5.46 (1H), 7.2~7.5 (5H)
4e[a]	CH_3	CH_3	9.30	6.66	5.75	5.70	5.12	3.90	1.38	1.32 (6H, s)
4f[b]	CH_3	Et	9.40	6.68	5.80	5.90, 5.93	5.10~5.35	3.98	1.40	1.91 (3H, m), 1.45, 1.55, 0.91 (3H, t)

[a] Mixture of two diastereoisomers.
[b] Mixture of four diastereoisomers.

Table 3. ^{1}H NMR spectral data of **5**.

Compounds			^{1}H NMR δ value (DMSO-d_6) ppm						
No.	R_1	R_2	CONH (1H, d)	Thiazole 5-H (1H, s)	7-H (1H, dd)	3-CH< (1H, s)	6-H (1H, d)	2-H (2H, br s)	R_1, R_2
5a[a]	H	H	9.52	6.80	5.88	5.72	5.16	3.92	4.59 (2H, br s)
5a-I	H	H	9.51	6.82	5.84	5.72	5.15	3.92	4.60 (2H, br s)
5a-II	H	H	9.50	6.80	5.86	5.72	5.14	3.90	4.60 (2H, br s)
5b[b]	H	CH_3	9.46, 9.51	6.78	5.8~6.0	5.73	5.17	3.94	4.62 (1H, m), 1.45 (3H, d)
5c[b]	H	Et	9.52	6.78, 6.99	5.60, 5.92	5.75	5.04~5.18	3.96	4.50 (1H, m), 1.82 (2H, m), 0.98 (3H, t)
5d[b]	H	Ph	9.56, 9.68	6.82, 6.86	5.90	5.65, 5.67	5.14, 5.19	3.92	7.3~7.6 (5H, br s), 5.58 (1H, s)
5e[a]	CH_3	CH_3	9.40	6.72	5.92	5.72	5.16	3.94	1.44 (6H, s)
5f[b]	CH_3	Et	9.44	6.69	5.88	5.72	5.15	3.93	5.06, 1.77, 1.40, 1.38, 0.85 (3H, t)

[a] Mixture of two diastereoisomers.
[b] Mixture of four diastereoisomers.

Experimental

NMR spectra were recorded at 90 MHz on a Jeol FX-90Q spectrometer and at 100 MHz on a Jeol MH100NMR spectrometer using tetramethylsilane as an internal standard. IR spectra were taken on a Hitachi 260-10 spectrophotometer. For column chromatography, silica gel (Wakogel C-200) was used. MP's of the cephalosporins are not accurately reproducible because of extensive decomposition.

General Procedure for the Preparation of 3-[4-(Carbamoyl Carboxymethylene)-1,3-dithietan-2-yl]-7β-[(*Z*)-2-(2-tritylamino-4-thiazolyl)-2-(*tert*-butoxycarbonylalkoxyimino)acetamido]-3-cephem-4-carboxylic Acid (**4**)

A solution of 3-(5-isothiazolyl)thiomethylcephem (**3**) (0.5 mmol) and triethylamine (250 μg) in DMSO (3 ml) and dioxane (10 ml) was stirred for 3~4 days at room temperature. After concentration, the residue was added to ice-water (10 ml) and acidified with 2 N HCl (5 ml). The aqueous solution was extracted with methyl ethyl ketone (MEK) (20 ml × 2). The combined extracts were washed with water (10 ml × 2), saturated brine (10 ml) and dried ($MgSO_4$) and then the solvent was evaporated under reduced pressure. The residue was fractionated by silica gel chromatography ($CHCl_3$-2-PrOH-HCOOH, 90:10:2) to give, after trituration with mixture of Et_2O and *n*-hexane, **4** as a powder.

General Procedure for the Preparation of 3-[4-(Carbamoyl Carboxymethylene)-1,3-dithietan-2-yl]-7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-hydroxycarbonylalkoxyimino)acetamido]-3-cephem-4-carboxylic Acid (**5**)

TFA (6 ml) was added to a mixture of **4** (0.3 mmol) in anisole (0.5 ml) under ice-cooling, and then the mixture was stirred for 1 hour at 19~21°C. After removal of the TFA under reduced pressure without heating, the residue was triturated with Et_2O. The collected precipitate was added to a mixture of TFA (6 ml) and water (2 ml) under ice-cooling. After being stirred at 19~21°C for 1 hour, the TFA and water were evaporated under reduced pressure. The residue was triturated with Et_2O to give **5** as a powder.

Preparation of Diphenylmethyl 3-[4-(Carbamoyl Diphenylmethoxycarbonylmethylene)-1,3-dithietan-2-yl]-7β-[(*Z*)-2-(2-tritylamino-4-thiazolyl)-2-(*tert*-butoxycarbonylmethoxyimino)acetamido]-3-cephem-4-carboxylate (**6a-I** and **6a-II**)

Diphenyldiazomethane was slowly added to a solution of **4a** (2.8 g, 3 mmol) in CH_2Cl_2 (200 ml) with stirring at room temperature until the evaporation of N_2 ceased. After concentration, the product was fractionated by silica gel column chromatography (benzene-EtOAc, 3:1) to give, after being triturated with a mixture of Et_2O and *n*-hexane, the less polar isomer (**6a-I**, 680 mg yield 18%) and the more polar isomer (**6a-II**, 1.09 g yield 28.7%), as powder, respectively. **6a-I**: Rf 0.54 (benzene-EtOAc, 3:1); $[\alpha]_D^{20}$ −178.5° (*c* 1, CH_2Cl_2); IR (KBr) cm^{-1} 3350, 1785, 1720, 1680, 1630, 1490, 1365, 1260, 1085, 750, 695; ^1H NMR ($CDCl_3$) δ 1.38 (9H, s, *tert*-Bu), 3.90 (2H, ABq, J=18 Hz, 2-H), 4.74 (2H, s, –O–CH_2–), 5.06 (1H, d, J=5.7 Hz, 6-H), 5.82 (1H, s, 3-CH<), 5.90 (1H, dd, J=8.6 and 5.7 Hz, 7-H), 6.80 (1H, s, thiazole-H), 6.94 (1H, s, Ph_2CH), 6.98 (1H, s, Ph_2CH), 7.1~7.5 (35H, m, phenyl-H), 8.88 (1H, d, J=8.6 Hz, CONH). **6a-II**: Rf 0.35 (benzene-EtOAc, 3:1); $[\alpha]_D^{20}$ −40.4° (*c* 1, CH_2Cl_2); IR (KBr) cm^{-1} 3360, 1785, 1722, 1680, 1635, 1490, 1365, 1265, 1090, 750, 695; ^1H NMR ($CDCl_3$) δ 1.32 (9H, s, *tert*-Bu), 3.90 (2H, ABq, J=18 Hz, 2-H), 4.75 (2H, s; –O–CH_2–), 5.07 (1H, d, J=5.7 Hz, 6-H), 5.84 (1H, s, 3-CH<), 5.95 (1H, dd, J=8.6 and 5.7 Hz, 7-H), 6.82 (1H, s, thiazole-H), 6.93 (1H, s, Ph_2CH), 6.99 (1H, s, Ph_2CH), 7.1~7.5 (35H, m, phenyl-H), 8.68 (1H, d, J=8.6 Hz, CONH).

Preparation of 3-[4-(Carbonyl Carboxymethylene)-1,3-dithietan-2-yl]-7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-hydroxycarbonylmethoxyimino)acetamido]-3-cephem-4-carboxylic Acid (**5a-I** and **5a-II**)

The diastereoisomer (**5a-I**: $[\alpha]_D^{20}$ −293.5° (*c* 1, DMF)) was prepared from **6a-I** in 91% and the other isomer (**5a-II**: $[\alpha]_D^{20}$ −108.5° (*c* 1, DMF)) was prepared from **6a-II** in 93% as described in the general procedure for the preparation of **5**.

Acknowledgments

We are grateful to the staff of the Chemotherapy and Antibiotics Department for the measurements of antibacterial activities and to the members of the Physico Analytical Center of Yamanouchi Pharmaceutical Co., Ltd.

References

1) IWANAMI, M.; T. MAEDA, M. FUJIMOTO, Y. NAGANO, N. NAGANO, A. YAMAZAKI, T. SHIBANUMA, K. TAMAZAWA & K. YANO: Synthesis of new cephamycin derivatives and a novel rearrangement between isothiazolethioacetamides and 1,3-dithietanecarboxamides. Chem. Pharm. Bull. 28: 2629~2636, 1980
2) NAGANO, N.; K. NAKANO, T. SHIBANUMA, Y. MURAKAMI & R. HARA: Studies on β-lactam antibitics. I. Synthesis and *in vitro* anti-pseudomonal activity of 3-isothiazole-cephalosporin derivatives. J. Antibiotics 40: 173~181, 1987