CEPHALOSPORINS. III. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 7-VINYLENETHIOACETAMIDO CEPHALOSPORINS WITH A TETRAZOLO-PYRIDAZINE AT THE 3-POSITION

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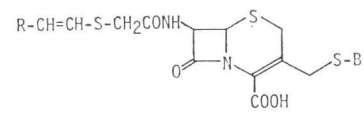
The synthesis and *in vitro* activity of 7-vinylenethioacetamido cephalosporins with a tetrazolo-pyridazine at the 3-position are described. These cephalosporins showed good activity against Gram-positive and Gram-negative bacteria. 7-[(*Z*)-β-Carboxyvinylenethioacetamido]-3-[(tetrazolo[1,5-b]pyridazin-8-amino-6-yl)-thiomethyl]-3-cephem-4-carboxylic acid (K 13176, **21**) was significantly more active *in vitro* and *in vivo* than cefazolin against Gram-negative bacteria.

An earlier paper[1] described the synthesis of 7-vinylenethioacetamido cephalosporins and their analogues with five-membered heterocycles at the 3-position. The antibacterial activity of these cephalosporins varied considerably depending on the functional group introduced in the 7-side chain, the stereochemistry of the double bond and the heterocycle at the 3-position. Of these, 7-[(*Z*)-β-cyanovinylenethioacetamido-3-(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl]-3-cephem-4-carboxylic acid (K 13101) was several times more active *in vitro* than cefazolin, but its *in vivo* activity was equal to or only slightly higher.

As an extension of our program to improve both the antibacterial activity and pharmacokinetic properties, we decided to pursue our initial approach in the 7-vinylenethioacetamido cephalosporins synthesizing a series of compounds with a tetrazolo[1,5-b]pyridazine at the 3-position[2].



R = CN, $CONH_2$, $COOR_1$ (R_1 = H, CH_3, C_2H_5)

B = Tetrazolo[1,5-b]pyridazine

This paper describes the synthesis of these new cephalosporins, the preparation of the new bicyclic heteroaromatic thiols and the results of our structure-activity studies.

Chemistry

Some of the side chain acids (R = CN, $CONH_2$, COOH) have already been reported in our previous paper[1]. Our first approach to synthesizing the others (R = $COOCH_3$, $COOC_2H_5$) by addition of thioglycolic acid to methyl or ethyl propiolate, according to the procedure previously described[1], afforded only a mixture of (*Z*)- and (*E*)-isomers (60: 40), from which the pure (*Z*)-isomer was obtained by crystallization of the dicyclohexylamine salt and subsequent release of the free acid by treatment with H_3PO_4. This process suffered from poor stereoselectivity and yield (about 28%).

Better results were achieved with an alternative synthesis (Scheme 1), in which *tert*-butylthioglycolate was added stereoselectively to propiolic acid, affording only the (*Z*)-isomer (**1**) in a high yield, which was then converted to the acyl chloride (**2**) by treatment with phosphorus pentachloride in ethyl ether. Reaction with sodium methoxide or ethoxide in a mixture of ethyl ether and methanol or ethanol gave **3** or **4** respectively. The final step of the process was to remove the *tert*-butyl group with trifluoroacetic acid to obtain the free acids **5** or **6**.

Bicyclic heteroaromatic thiols used in our study were prepared as reported in Scheme 2 and are listed in Table 1.

Scheme 1. Substituted vinylenethioacetic acids.

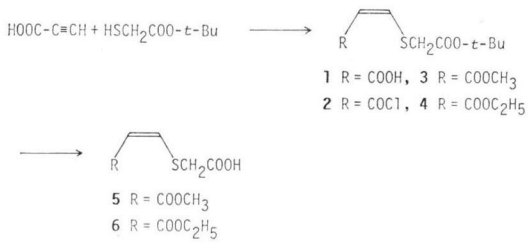


Table 1. Bicyclic heteroaromatic thiols (**11**).

Compound	Mp (°C)	Yield[a]	Formula
11a[11]	142~144	97	$C_4H_3N_5S$
11b	145 (dec.)	75	$C_5H_5N_5S$
11c	>300	90	$C_4H_4N_6S$
11d	230 (dec.)	80	$C_5H_6N_6S$
11e	218~220 (dec.)	80	$C_6H_6N_6O_2S$
11f	209~211 (dec.)	93	$C_5H_3N_5O_2S$
11g	185~187 (dec.)	70	$C_5H_4N_6OS$
11h	150 (dec.)	80	$C_4H_4N_6S$

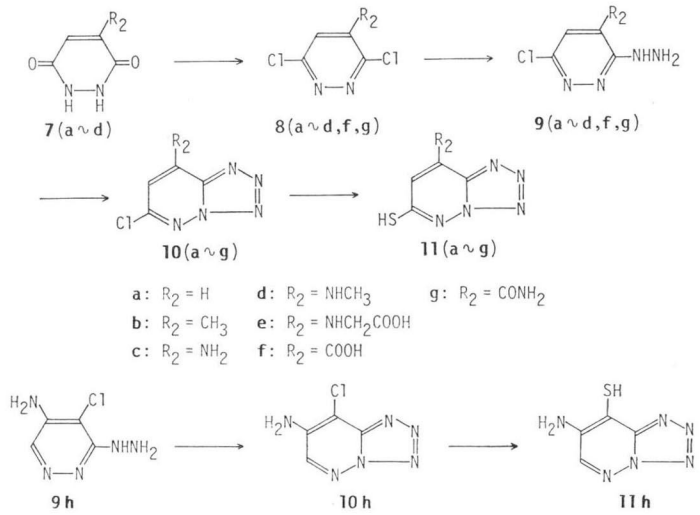
[a] Calculated from the corresponding chlorides (**10**)

The intermediates **8**~**11** were partly already known from the literature; the unknown ones were prepared following procedures in the literature, which were modified in some cases (see Experimental).

Refluxing 1,2-dihydropyridazin-3,6-dione **7** (**a**, **b**) with $POCl_3$ gave 3,6-dichloropyridazines **8** (**a**, **b**). In the case of **8c** we modified the synthesis reported in literature[3] and studied an alternative method in order to avoid the irritant 3,4,6-trichloropyridazine. For this purpose we started from 4-amino-1,2-dihydropyridazin-3,6-dione, and converted it to **8c** by heating with $POCl_3$ at 120°C in a sealed tube. Refluxing with $POCl_3$ gave only 4-amino-6-chloro-pyridazin-3(2*H*)-one.

By a similar procedure 4-methylamino-1,2-dihydropyridazin-3,6-dione was chlorinated with $POCl_3$ to give **8d** which proved identical to that described in the literature[4].

Scheme 2.



Oxidation of **8b** with $K_2Cr_2O_7$ in conc. H_2SO_4 gave **8f**[5], which was first converted to ethyl 3,6-dichloropyridazine-4-carboxylate by reaction with ethanol and conc. H_2SO_4, from which **8g**[6] was obtained by treatment with 28% aqueous ammonia.

Refluxing **8(a~d, f, g)** with hydrazine hydrate in ethanol gave **9a**[7], **9b**[8], **9c**[8], **9d**[4], **9f** and **9g**[6] respectively.

Reaction of **9** (**a~d, f, g**) with sodium nitrite in mineral acid gave **10a**[9], **10b**[8], **10c**[10], **10d, 10f** and **10g**. Treatment of **10c** with bromoacetic acid and NaH in DMSO gave **10e**.

Compounds **10** (**a~g**) were converted to the corresponding thiols **11** (**a**[11], **b~g**) by reaction with alkali hydrogen sulfide in water or ethanol (see Experimental).

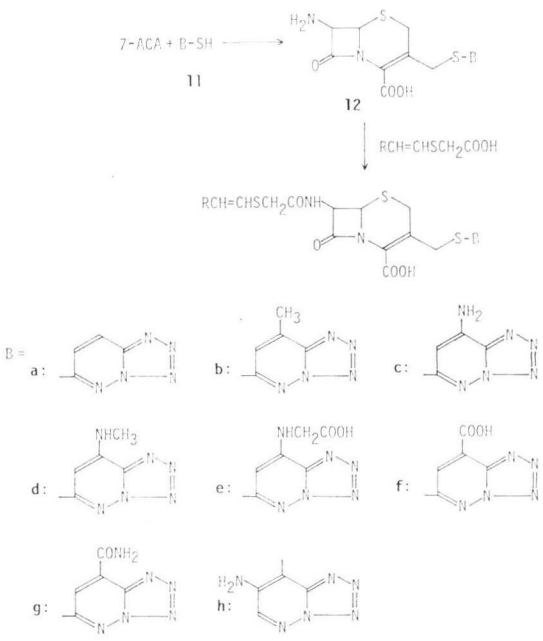
Cyclization of **9h** with sodium nitrite in hydrochloric acid gave **10h**[12], which was then converted to **11h** by reaction with potassium hydrogen sulfide.

Nucleophilic displacement of the C-3 acetoxy group of 7-ACA with the bicyclic thiols (**11**) was achieved in the usual manner[13], also outlined in the Experimental Section to give **12** (Table 2). The structure of these derivatives was established by their infrared spectra (β-lactam C=O absorption at 1770~1805 cm^{-1}) and UV maxima at 260~275 nm due to the cephem nucleus and that due to the heterobicyclic ring in the C-3 side chain.

The cephalosporins were prepared by acylating **12** with β-substituted vinylenethioacetic acids (Scheme 3). Acylation was carried out using mixed anhydride derived from pivaloyl chloride (method A). When a second carboxyl group was present in the acylating acid (R=COOH), this was protected as its *tert*-butyl ester, which was subsequently removed with trifluoroacetic acid-anisole (method B).

The cephalosporins synthesized are listed in Table 3. The purity of the cephalosporins, established by NMR, TLC, and analyses, was greater than 90%.

Scheme 3. Preparation of 3-substituted-7-β-vinylenethioacetamido cephalosporins.



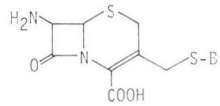
Antimicrobial Activity

The minimum inhibitory concentrations (MICs) of this series of cephalosporins against 3 strains of Gram-positive and 6 strains of Gram-negative bacteria were determined by the standard two-fold serial dilution method using diagnostic sensitivity test agar (Oxoid). The plates were inoculated with about 2×10^5 colony forming units using an automatic inoculator (Denley Tech. Ltd.). The results are the geometric average of two determinations and are compared with cefazolin (CEZ) (Tables 4~6).

Table 4 shows the effects on biological activity of altering the functional group at the 7-side chain, while the tetrazolo[1,5-b]pyridazine, unsubstituted or substituted with an amino group, was introduced at the 3-position. Comparison of some (*E*) and (*Z*)-isomers is reported too.

In line with our previous observations[1] (*Z*)-isomers (**19, 20** and **21**) were more effective against both

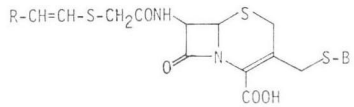
Table 2. 3-Substituted 7-aminocephalosporanic acids (**12**).



Compound	Yield %	Mp (°C, dec.)	λ_{max} nm (ε)	Formula[d]
12b	60	250	242 (22114)[a]	$C_{13}H_{13}N_7O_3S_2$
12c	75	250	262 (21874)[b]	$C_{12}H_{12}N_8O_3S_2$
12d	80	260	269 (23587)[c] 296 (16585)	$C_{13}H_{14}N_8O_3S_2$
12e	43	265	270 (20299)[a] 300 (18546)	$C_{14}H_{14}N_8O_5S_2$
12f	77	245	247 (21737)[a] 320 (5649)	$C_{13}H_{11}N_7O_5S_2$
12g	66	228~230	249 (21679)[b] 270s 332 (4432)	$C_{13}H_{12}N_8O_4S_2$
12h	50	230	264 (13429)[a] 350 (4641)	$C_{12}H_{12}N_8O_3S_2$

[a] Determined in pH 7.4 phosphate buffer.
[b] Determined in 1% $NaHCO_3$ solution.
[c] Determined in 1% NaOH solution.
[d] All compounds were analysed for C, H, N, S. Analytical results are coincident with the calculated value within $\pm$0.50% deviation.

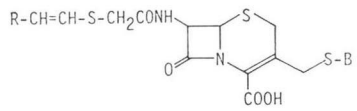
Table 3. 3-Substituted-7-β-vinylenethioacetamido cephalosporins.



Compound	R	Configuration	B[b]	Method	IR (β-lactam) KBr, cm^{-1}	Formula[a]
13	NC	*E*	c	A	1770	$C_{17}H_{15}N_9O_4S_3$
14	H_2NCO	*E*	c	A	1760	$C_{17}H_{17}N_9O_5S_3$
15	HOOC	*E*	c	B	1770	$C_{17}H_{16}N_8O_6S_3$
16	NC	*Z*	a	A	1770	$C_{17}H_{14}N_8O_4S_3$
17	H_2NCO	*Z*	a	A	1780	$C_{17}H_{16}N_8O_5S_3$
18	HOOC	*Z*	a	B	1780	$C_{17}H_{15}N_7O_6S_3$
19	NC	*Z*	c	A	1770	$C_{17}H_{15}N_9O_4S_3$
20	H_2NCO	*Z*	c	A	1765	$C_{17}H_{17}N_9O_5S_3$
21	HOOC	*Z*	c	B	1760	$C_{17}H_{16}N_8O_6S_3$
22	NC	*Z*	b	A	1775	$C_{18}H_{16}N_8O_4S_3$
23	NC	*Z*	d	A	1770	$C_{18}H_{17}N_9O_4S_3$
24	NC	*Z*	e	A	1760	$C_{19}H_{17}N_9O_6S_3$
25	NC	*Z*	f	A	1765	$C_{18}H_{14}N_8O_6S_3$
26	NC	*Z*	g	A	1770	$C_{18}H_{15}N_9O_5S_3$
27	NC	*Z*	h	A	1760	$C_{17}H_{15}N_9O_4S_3$
28	H_3COOC	*Z*	c	A	1770	$C_{18}H_{13}N_8O_6S_3$
29	H_5C_2OOC	*Z*	c	A	1770	$C_{19}H_{20}N_8O_6S_3$
30	HOOC	*Z*	h	B	1780	$C_{17}H_{16}N_8O_6S_3$

[a] See footnote d to Table 2.
[b] See Scheme 3.

Table 4. *In vitro* activity of 7-[(*E*)- and (*Z*)-β-substituted vinylenethioacetamido]cephalosporins.



Compound	R	Configuration	B[b]	MIC (μg/ml)[a]								
				S. a.	*S. a.* (R)	*S. p.*	*E. c.*	*E. a.*	*K. p.*	*S. t.*	*Sh. s.*	*P. m.*
13	NC	*E*	c	0.012	0.1	0.006	2.2	2.2	0.14	1.6	17.7	6.2
14	H_2NCO	*E*	c	0.035	0.14	0.006	1.6	0.8	0.14	0.8	3.1	6.2
15	HOOC	*E*	c	0.4	1.6	0.1	1.6	0.4	0.2	0.57	8.8	0.2
16	NC	*Z*	a	0.012	0.1	≤0.006	0.8	0.57	0.2	0.2	0.8	0.8
17	H_2NCO	*Z*	a	0.025	0.14	≤0.006	0.57	0.4	0.2	0.2	1.6	0.8
18	HOOC	*Z*	a	0.05	0.8	0.025	0.8	0.4	0.2	0.2	1.6	0.2
19	NC	*Z*	c	0.006	0.05	≤0.006	0.4	0.4	0.05	0.2	1.6	0.4
20	H_2NCO	*Z*	c	0.017	0.1	≤0.006	0.8	0.57	0.2	0.4	1.6	0.8
21	HOOC	*Z*	c	0.05	0.57	0.015	0.28	0.2	0.05	0.1	0.8	0.05
CEZ				0.05	0.57	0.05	1.6	1.6	0.8	1.6	1.6	6.2

[a] Organisms selected for inclusion in this Table are: *S.a.*, *Staphylococcus aureus* Smith (penicillin G sensitive); *S.a.* (R), *Staphylococcus aureus* 39/2 (penicillin G resistant); *S.p.*, *Streptococcus pyogenes* C 203; *E. c.*, *Escherichia coli* G; *E. a.*, *Enterobacter aerogenes* ATCC 8308; *K.p.*, *Klebsiella pneumoniae* ATCC 10031; *S.t.*, *Salmonella typhi* Watson; *Sh. s.*, *Shigella sonnei* ATCC 11060; *P.m.*, *Proteus mirabilis* ATCC 9921.

[b] See Scheme 3.

Gram-positive and Gram-negative bacteria than the corresponding (*E*) isomers (**13**, **14** and **15**). Substitution of the –CN group (**13**, **16** and **19**) either with $-CONH_2$ (**14**, **17** and **20**) or –COOH (**15**, **18** and **21**) led in general to reduced activity against Gram-positive bacteria. The only exception was *Streptococcus pyogenes* C 203, against which substitution of the –CN group with $-CONH_2$ maintained the activity constant (**14**, **17**, **20**). The effect on the activity against Gram-negative bacteria was variable.

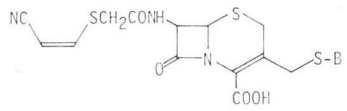
In the series of unsubstituted tetrazolo[1,5-b]pyridazine (**16**, **17** and **18**) the variation of the activity against Gram-negative bacteria was less significant. Conversely, in the series of 8-amino-tetrazolo-[1,5-b]pyridazine (**19**, **20** and **21**) substitution of the –CN group with $-CONH_2$ resulted in reduction of the activity against Gram-negative bacteria, while substitution with –COOH improved the activity. Derivatives containing a –COOH group (**15**, **18** and **21**) showed significantly greater activity against *Proteus mirabilis* ATCC 9921. Compound **19** was the most active against Gram-positive bacteria, while **21** resulted the most active against Gram-negative.

In view of their activity, derivatives containing the –CN group were selected for further modifications at the 3-position. Table 5 summarizes the activity of all the 7-(*Z*)-β-cyanovinylenethioacetamido cephalosporins synthesized. The modifications on the heterobicyclic ring had various effects on the activity. Introduction of an amino group (c) enhanced (with some exceptions) the activity against both Gram-positive and Gram-negative bacteria, while the other modifications (**22**～**26**) dramatically reduced the activity especially against Gram-negative bacteria. **27** was less active than its isomer **19**.

Table 6 sets out the activity of compounds **28** and **29** in which the –COOH group of the 7-side chain was esterified. Esterification of –COOH enhanced the activity against Gram-positive bacteria, but reduced the activity against Gram-negative ones. **30** was less active than its isomer **21**.

Of the compounds synthesized, K 13102 (**19**) and K 13176 (**21**) proved to be the most active on the

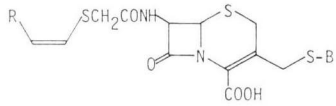
Table 5. *In vitro* activity of 7-(*Z*)-β-cyanovinylenethioacetamido cephalosporins.



Compound	B[b]	MIC (μg/ml)[a]								
		S. a.	*S. a.* (R)	*S. p.*	*E. c.*	*E. a.*	*K. p.*	*S. t.*	*Sh. s.*	*P. m.*
16	a	0.012	0.1	≤0.006	0.8	0.57	0.2	0.2	0.8	0.8
22	b	≤0.006	0.1	≤0.006	1.6	1.6	0.4	0.8	12.5	1.6
19	c	0.006	0.05	≤0.006	0.4	0.4	0.05	0.2	1.6	0.4
23	d	0.012	0.1	≤0.006	3.1	6.2	0.2	1.6	12.5	1.6
24	e	0.07	0.4	0.006	6.2	6.2	3.1	1.6	25	3.1
25	f	0.05	0.28	≤0.006	12.5	1.6	12.5	0.8	35.4	0.4
26	g	≤0.006	0.2	≤0.006	3.1	3.1	0.8	0.8	25	3.1
27	h	0.1	0.4	0.006	1.1	1.6	0.4	0.4	4.4	0.8
CEZ		0.05	0.57	0.05	1.6	1.6	0.8	1.6	1.6	6.2

[a] See footnote a to Table 4.
[b] See Scheme 3.

Table 6. *In vitro* activity of 7-(*Z*)-β-carboxy or carboalkoxyvinylenethioacetamido cephalosporins.



Compound	R	B[b]	MIC (μg/ml)[a]								
			S. a.	*S. a.* (R)	*S. p.*	*E. c.*	*E. a.*	*K. p.*	*S. t.*	*Sh. s.*	*P. m.*
21	HOOC	c	0.05	0.57	0.015	0.28	0.2	0.05	0.1	0.8	0.05
28	H_3COOC	c	0.006	0.2	≤0.0015	2.2	2.2	0.14	0.8	6.2	3.1
29	H_5C_2OOC	c	0.012	0.2	0.001	8.8	4.4	0.4	2.2	35.4	1.1
30	HOOC	h	1.6	6.2	0.8	0.8	0.57	0.28	0.28	1.6	0.8
CEZ			0.05	0.57	0.05	1.6	1.6	0.8	1.6	1.6	6.2

[a] See footnote a to Table 4.
[b] See Scheme 3.

Table 7. *In vivo* activity of K 13176 (**21**) and cefazolin in acute systemic infection in mice[a].

Challenge organism	ED_{50} in mg/kg (Confidence limits for P=0.95)		
	K 13176	Cefazolin	Potency ratio *vs* cefazolin
Staphylococcus aureus Smith	0.29 (0.24~0.34)	0.22 (0.18~0.27)	0.78 (0.60~1.008)
Streptococcus pyogenes C 203	0.47 (0.39~0.57)	0.58 (0.48~0.70)	1.24 (0.96~1.59)
Escherichia coli G	0.32 (0.20~0.49)	7.53 (6.22~9.12)	23.7 (15.19~36.97)
Proteus mirabilis ATCC 9921	0.70 (0.47~0.90)	14.82 (12.47~17.81)	21.1 (13.79~32.31)
Proteus vulgaris X 20	1.66 (1.29~2.14)	23.97 (20.07~28.63)	14.42 (10.81~19.23)
Klebsiella pneumoniae ATCC 10031	0.49 (0.38~0.61)	5.62 (4.63~6.85)	11.54 (8.40~15.68)
Salmonella typhi Watson	0.10 (0.06~0.15)	5.78 (4.61~7.01)	53.53 (35.5~80.7)
Haemophilus influenzae 10479	1.44 (1.1~1.89)	9.26 (7.35~11.67)	6.42 (4.38~9.40)
Escherichia coli G R^+ TEM	11.76 (9.07~15.24)	40.35 (30.67~53.09)	3.43 (2.36~4.99)

[a] Experiments carried out with groups of 14~28 mice per dose.

whole. Compound **19** showed good activity against both Gram-positive and Gram-negative bacteria. In fact, it was 6~8 times more active than cefazolin against Gram-positive bacteria and 4~16 times against Gram-negative ones; the exception was *Shigella sonnei* ATCC 11060, against which it was as active as cefazolin. **21** was less active against Gram-positive than **19**. Its activity was only equal to that of cefazolin against *Staphylococci* and 3 times more active against *Streptococci*; but it showed greater activity against Gram-negative organisms. It was about 120 times more active than cefazolin against *Proteus mirabilis* ATCC 9921.

21 was selected for further *in vitro* studies including clinical isolates of *E. coli, Klebsiella* and *Proteus mirabilis* (30 strains of each). The geometric means of the MICs on these organisms were respectively 0.63, 0.3 and 0.13 μg/ml, while the corresponding MIC values for cefazolin were 1.74, 2.6 and 5.38 μg/ml. Against *Haemophilus influenzae* **21** was twice as active as cefuroxime and 35 times more active than cefazolin.

The most active compounds were tested *in vivo* in mice against experimental infections with *Staphylococcus aureus* Smith and *Escherichia coli* G according to the following method: male albino CD-1 COBS mice (18~20 g) were infected intraperitoneally with bacterial suspensions in amounts corresponding to the LD_{99}. Treatments were given subcutaneously immediately after the infection and 3 hours later. A complete balanced block design was followed with random assignment of the drugs (2~4 groups of 7 mice per dose). The animals were kept under observation for 5 days when they had been infected with Gram-negative bacteria and for 7 days when they had been infected with Gram-positives. From the survival rates the median effective dose (ED_{50}) and the potency ratios between the drugs were calculated by probit analysis[14].

Of the compounds selected for *in vivo* evaluation compound **21** proved 23 times more active than cefazolin against *Escherichia coli* G and therefore was studied more widely, in comparison with cefazolin, in mice infected with 7 other bacteriae strains. The results are reported in Table 7.

Against *H. influenzae, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris* and *S. typhi* Watson, **21** was 6 to 53 times more potent than CEZ. Against Gram-positive bacteria the activity of **21** was equal to that of CEZ.

The superior *in vitro* activity against Gram-negative bacteria, in comparison with cefazolin, is therefore confirmed by the higher *in vivo* therapeutic efficacy.

Experimental

Infrared spectra were recorded on a Perkin-Elmer spectrometer (model 125). The NMR spectra were determined on either a Perkin-Elmer R-24 B (60 MHz) or a Bruker HX-90 (90 MHz) spectrometer using tetramethylsilane as internal standard, and chemical shifts are reported in parts per million (δ) relative to Me_4Si. Melting points were established on a Büchi melting point apparatus and are not corrected. Melting points of the cephalosporins are not accurately reproducible because of extensive decomposition.

(*Z*)-β-Methoxycarbonylvinylenethioacetic Acid (**5**)

A solution of 70% thioglycolic acid (6.4 ml, 60 mmole) in 2 N NaOH (30 ml) and water (23.6 ml) was added dropwise, with stirring, to an ice-cold solution of methylpropiolate (5.3 g, 63 mmole) in acetone (76 ml) and water (38 ml). After stirring for 1 hour at 0°C and an additional hour at 10°C the acetone was removed *in vacuo*. Water was added and the reaction mixture was adjusted to pH 9~9.5 with a few drops of 2 N aqueous NaOH. The aqueous solution was washed with ethyl acetate, acidified with 20% H_2SO_4, and extracted with ethyl acetate. The organic layer was washed with saturated aqueous

NaCl solution, dried (Na_2SO_4) and evaporated *in vacuo*. To the residual oil dissolved in ethyl ether (100 ml) a stoichiometric amount of dicyclohexylamine was added dropwise.

The precipitated salt was collected, washed twice with ethyl ether and dried *in vacuo* to give 17.2 g of the dicyclohexylamine salt, which consisted of a mixture of (*Z*)- and (*E*)-isomers (60: 40). Pure (*Z*)-isomer (33%) was obtained by fractionated crystallization of the dicyclohexylamine salt from ethyl ether; the purification process was checked by TLC (ethyl ether - petroleum ether - formic acid, 150: 50: 5); mp 137~139°C; NMR ($CDCl_3$): δ 0.5~2.1 (20 H, m, CH_2 on dicyclohexylamine), 2.7~3.2 (2H, m, CH on dicyclohexylamine), 3.3 (2H, s, SCH_2), 3.65 (3H, s, OCH_3), 5.7 (1H, d, J=10 Hz, OC–CH=), 7.3 (1H, d, J=10 Hz, =CHS), 8.6 (2H, br-s, NH_2^+).

Anal. Calcd. for $C_{18}H_{31}NO_4S$: C, 60.47; H, 8.74; N, 3.92; S, 8.97.
Found: C, 60.54; H, 8.79; N, 3.89; S, 8.78.

The solution of the salt (2.8 g) in water (50 ml), stratified with 70 ml of ethyl acetate at 5°C, was acidified by dropwise addition of 40% H_3PO_4 until pH 2 and extracted three times with ethyl acetate; the combined extracts were washed with saturated aqueous NaCl solution, dried (Na_2SO_4) and evaporated *in vacuo* to yield 1.2 g (87%) of **5**; mp 73~76°C; IR (Nujol): 1690~1720, 1580 cm^{-1}; NMR (acetone-d_6): δ 3.45 (2H, s, SCH_2), 3.85 (3H, s, OCH_3), 5.85 (1H, d, J=10 Hz, OC–CH=), 7.35 (1H, d, J=10 Hz, =CH–S), 8.0 (1H, br-s, COOH).

Anal. Calcd. for $C_6H_8O_4S$: C, 36.57; H, 4.90; S, 19.52.
Found: C, 36.41; H, 4.80; S, 19.37.

(*Z*)-β-Ethoxycarbonylvinylenethioacetic Acid (**6**)

A solution of *tert*-butylthioglycolate (5 g, 33.8 mmole) in 95% ethanol (17 ml) was added dropwise, with stirring, to an ice-cold solution of propiolic acid (2.36 g, 33.8 mmole) in 2 N KOH (16.9 ml) and water (8.5 ml). The reaction mixture was stirred for 2 hours at 0~5°C and subsequently for 1 hour at room temperature, maintaining the pH at 8~8.5 by adding KOH as necessary. The solution was then cooled to 0~5°C and carefully acidified with 2 N HCl (17 ml). After stirring for 1 hour the solid precipitate was collected, washed with cold water and dried *in vacuo* to give 6.45 g (87%) of **1**; mp 113~115°C; NMR ($CDCl_3$): δ 1.5 (9H, s, –COO-*t*-Bu), 3.4 (2H, s, SCH_2), 6.0 (1H, d, J=10 Hz, HOOC–CH=), 7.4 (1H, d, J=10 Hz, =CH–S), 8.5 (1H, s, –COOH).

Anal. Calcd. for $C_9H_{14}O_4S$: C, 49.52; H, 6.46; S, 14.68.
Found: C, 49.50; H, 6.50; S, 14.75.

To a stirred suspension of **1** (1.09 g, 5 mmole) in ethyl ether (50 ml), cooled to 0°C, PCl_5 (1.04 g, 5 mmole) was added portionwise and the mixture was stirred for 2 hours at 0°C. After evaporation *in vacuo* below 40°C, the residue was taken up with benzene and evaporated again *in vacuo*, leaving **2** quantitatively as an almost colorless oil, which was used for the next step without further purification.

To a stirred solution of sodium ethoxide (0.340 g, 5 mmole) in dry ethyl ether (30 ml) and dry ethanol (10 ml), cooled to 0°C, a solution of **2** (1.18 g, 5 mmole) in dry ethyl ether (20 ml) was added dropwise.

The mixture was stirred for 15 minutes at 0~5°C and then evaporated *in vacuo* without heating. The residue was taken up with ethyl ether (30 ml); the organic layer was washed twice with water, dried (Na_2SO_4) and evaporated *in vacuo* to give **4** quantitatively as a viscous oil; NMR (acetone-d_6): δ 1.3 (3H, t, $-CH_3$), 1.5 (9H, s, –COO-*t*-Bu), 3.4 (2H, s, SCH_2), 4.2 (2H, q, $-CH_2O$), 5.9 (1H, d, J=10 Hz, OC–CH=), 7.4 (1H, d, J=10 Hz, =CH–S).

Anal. Calcd for $C_{11}H_{18}O_4S$: C, 53.63; H, 7.36; S, 13.01.
Found: C, 53.52; H, 7.40; S, 12.98.

A mixture of the above ester **4** (1.2 g, 5 mmole) and trifluoroacetic acid (10 ml), cooled to 0~5°C, was stirred for 30 minutes. The end of the reaction was determined by TLC (benzene - ethyl acetate - CH_3COOH - acetone, 130: 25: 15: 60). The reaction mixture was then evaporated *in vacuo* below 35°C to remove trifluoroacetic acid.

The resulting residue was taken up with benzene and evaporated again *in vacuo*. This treatment was repeated three times, yielding 0.9 g (95%) of **6** as a waxy solid; NMR (acetone-d_6): δ 1.3 (3H, t, $-CH_3$), 3.4 (2H, s, SCH_2), 4.2 (2H, q, $-CH_2O$), 5.9 (1H, d, J=10 Hz, OC–CH=), 7.4 (1H, d, J=10 Hz, =CH–S), 8.7 (1H, br-s, –COOH).

Anal. Calcd for $C_7H_{10}O_4S$: C, 44.20; H, 5.29; S, 16.85.
Found: C, 44.32; H, 5.30; S, 16.79.

By a similar procedure **2** was first reacted with sodium methoxide in a mixture of methanol and ethyl ether to give **3**, and then converted to **5** (95% yield based on **2**), which was identical to that described above.

4-Amino-3,6-dichloropyridazine (**8c**)

A mixture of 4-amino-1,2-dihydropyridazine-3,6-dione (1.27 g, 10 mmole) and $POCl_3$ (37 ml) was heated for 15 hours at 120°C in a sealed tube. The excess of $POCl_3$ was removed *in vacuo* at 70°C. The residue was taken up with water (20 ml), stirred for 30 minutes at 50~60°C and filtered hot. The filtrate was cooled to 0~5°C and adjusted to pH 3.5 with 35% NaOH and stirred for 15 hours at 0~5°C. The solid obtained was filtered, suspended in water (15 ml), made alkaline with 20% NH_4OH and stirred for 1 hour at 0~5°C. The precipitate was collected, washed with water and dried *in vacuo* at 70°C to give 1.23 g (75.5%) of **8c**: mp 204~205°C (Ref. 204~205°C[8]).

Anal. Calcd. for $C_4H_3Cl_2N_3$: C, 29.29; H, 1.84; Cl, 43.24; N, 25.62.
Found: C, 29.33; H, 1.71; Cl, 43.01; N, 25.71.

3-Hydrazino-4-carboxy-6-chloro-pyridazine (**9f**)

A mixture of 3,6-dichloro-4-carboxy-pyridazine[5] (1.93 g, 10 mmole) and 98% hydrazine hydrate (2.16 g, 43.2 mmole) in dry ethanol (15 ml) was refluxed, with stirring, for 1 hour. After cooling to 5°C, the precipitate was collected and washed with cold ethanol. The solid was then suspended in water (10 ml), adjusted to pH 2 with 23% HCl, stirred for 1 hour at 0~5°C and filtered. The crude product was dissolved in hot water (30 ml), treated with charcoal and filtered. The filtrate was cooled in an ice bath, the resulting precipitate was collected and dried *in vacuo* at 80°C to give 1.76 g (93.4%) of **9f**; mp 198~201°C (dec.); NMR (DMSO-d_6): δ 7.8 (1H, s, 5-H on pyridazine ring), 9.2 (4H, br-s, –COOH, $-NHNH_2$).

Anal. Calcd. for $C_5H_5ClN_4O_2$: C, 31.84; H, 2.67; Cl, 18.80; N, 29.71.
Found: C, 31.64; H, 2.64; Cl, 18.53; N, 29.32.

6-Chloro-8-carboxy-tetrazolo[1,5-b]pyridazine (**10f**)

To an ice-cold suspension of 3-hydrazino-4-carboxy-6-chloro-pyridazine (1.88 g, 10 mmole) in a mixture of 35% HCl (2.04 ml) and water (30 ml) a solution of sodium nitrite (0.86 g, 12.5 mmole) in water (3 ml) was added dropwise in 10 minutes. After stirring for 2 hours at 5~10°C, the separated precipitate was collected, washed with cold water and dried *in vacuo* at 80°C to give 1.94 g (97%) of **10f**; mp 222~223°C (dec.); IR (Nujol): 1730 cm^{-1}; NMR (DMSO-d_6): δ 8.33 (1H, s, 7-H on pyridazine ring).

Anal. Calcd. for $C_5H_2ClN_5O_2$: C, 30.09; H, 1.00; Cl, 17.76; N, 35.09.
Found: C, 29.95; H, 0.98; Cl, 17.58; N, 35.15.

By a similar procedure **10d** and **10g** were prepared and the data for each compound are as follows:
10d; mp 244~246°C (dec.); IR (KBr): 3360, 3260, 2890, 1645, 1088, 980, 720 cm^{-1}.

Anal. Calcd. for $C_5H_5ClN_6$: C, 32.53; H, 2.73; Cl, 19.21; N, 45.53.
Found: C, 32.49; H, 2.77; Cl, 19.22; N, 45.54.

10g; mp 226°C (dec.); IR (KBr): 3410, 3240, 3180, 1700, 1680, 1620, 1080, 790 cm^{-1}.

Anal. Calcd. for $C_5H_3ClN_6O$: C, 30.24; H, 1.52; Cl, 17.85; N, 42.32.
Found: C, 30.11; H, 1.44; Cl, 17.69; N, 42.10.

6-Chloro-8-carboxymethylamino-tetrazolo[1,5-b]pyridazine (**10e**)

To an ice-cold solution of **10c**[10] (17 g, 0.1 mole) in dry DMSO (250 ml) a 50% oil suspension of NaH (0.3 mole) was added portionwise with stirring. When addition was complete, the temperature was allowed to rise to room temperature and a solution of bromoacetic acid (13.9 g, 0.1 mole) in DMSO (50 ml) was added dropwise. After stirring for 48 hours at room temperature, the solvent was removed *in vacuo* at 0.5~1 mmHg. The residue was taken up with water (200 ml); the undissolved material was filtered off and discarded. The filtrate was extracted with benzene (3×50 ml), the aqueous phase was adjusted to pH 2.5 with 37% HCl and the resulting precipitate was collected, washed with water and crystallized from water to give 13.69 g (60%) of **10e**; mp 241~242°C (dec.); IR (KBr): 3380, 3340, 3080, 1720, 1620, 1080 cm^{-1}.

Anal. Calcd. for $C_6H_5ClN_6O_2$: C, 31.52; H, 2.20; Cl, 15.51; N, 36.76.
Found: C, 31.33; H, 2.16; Cl, 15.54; N, 36.82.

6-Mercapto-8-methylamino-tetrazolo[1,5-b]pyridazine (**11d**)

A stirred solution of **10d** (10 g, 54 mmole) and KSH (9.6 g, 128 mmole) in dry ethanol (100 ml) was refluxed for 5 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in water (150 ml). The solution was clarified with activated carbon, filtered and the filtrate acidified to pH 1~2 with conc.HCl to precipitate **11d**, which was collected by filtration and washed with water.

The crude product was dissolved in 5% aqueous $KHCO_3$ solution (150 ml), the undissolved material was filtered and discarded. The filtrate was acidified with conc.HCl to give 8.0 g (80%) of pure **11d**; mp 230°C (dec.); IR (KBr): 3250, 2475 cm^{-1}; UV (1% $NaHCO_3$ solution) λ_{max} 269 nm (ε, 20153); NMR (DMSO-d_6); δ 2.98 (3H, d, J=3 Hz, $-CH_3$), 4.10 (1H, br, –SH), 6.56 (1H, s, 7-H on pyridazine ring), 8.60 (1H, br, J=3 Hz, –NH).

Anal. Calcd. for $C_5H_6N_6S$: C, 32.96; H, 3.32; N, 46.13; S, 17.59.
Found: C, 32.99; H, 3.28; N, 46.38; S, 17.46.

By a similar procedure **11e** and **11h** were prepared and the data for each compound are as follows:

11e; mp 218~220°C (dec.); IR (KBr); 3280~3100, 3080~3040, 3000~2300, 2920~2850, 2570, 1720, 1610~1570, 1440~1380 cm^{-1}; UV (1% $NaHCO_3$ solution) λ_{max} 269 nm (ε, 23300); NMR (DMSO-d_6): δ 4.30 (4H, br, J=5.5 Hz, CH_2, –SH, –COOH), 6.94 (1H, s, 7-H on pyridazine ring), 8.95 (1H, t, J=5.5 Hz, –NH).

Anal. Calcd. for $C_6H_6N_6O_2S$: C, 31.85; H, 2.67; N, 37.15; S, 14.17.
Found: C, 31.67; H, 2.68; N, 36.98; S, 13.71.

11h; mp 150°C (dec.); IR (KBr): 3370, 3310, 2470, 1640 cm^{-1}.

Anal. Calcd. for $C_4H_4N_6S$: C, 28.56; H, 2.39; N, 49.98; S, 19.06.
Found: C, 28.38; H, 2.32; N, 49.67; S, 18.77.

6-Mercapto-8-carboxy-tetrazolo[1,5-b]pyridazine (**11f**)

To a stirred solution of 75% NaSH (1.93 g, 25.9 mmole) in water (35 ml) 6-chloro-8-carboxy-tetrazolo[1,5-b]pyridazine (1.99 g, 10 mmole) was quickly added, under N_2 stream, and the mixture was vigorously stirred for 90 minutes at 20~25°C. The undissolved material was filtered off and discarded; the filtrate was cooled to 0~5°C and adjusted to pH 1~2 with 23% HCl. The suspension was stirred for 30 minutes at 0~5°C, the solid was collected, washed with water (20 ml) and dried *in vacuo* at 50°C to give 2.0 g (93%) of **11f**; mp 209~211°C (dec.); IR (KBr): 2540, 1725 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 258 nm (ε, 20683); NMR (DMSO-d_6): δ 6.06 (2H, br, –SH and –COOH), 8.61 (1H, s, 7-H on pyridazine ring).

Anal. Calcd. for $C_5H_3N_5O_2S$: C, 30.45; H, 1.53; N, 35.52; S, 16.26.
Found: C, 30.41; H, 1.52; N, 35.61; S, 16.19.

By a similar procedure **11b**, **11c** and **11g** were prepared and the data for each compound are as follows:

11b; mp 145°C (dec.); IR (KBr): 3060, 2920~2850, 1600~1565, 1450 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 253 nm (ε, 20650), 275 (ε, 7300), 334 (ε, 4320); NMR (Pyridine-d_6) δ: 2.50 (3H, d, J=1.5 Hz, CH_3), 7.72 (1H, q, J=1.5 Hz, 7-H on pyridazine ring).

Anal. Calcd. for $C_5H_5N_5S$: C, 35.97; H, 3.01; N, 41.89; S, 19.17.
Found: C, 35.83; H, 2.96; N, 41.83; S, 18.89.

11c; mp>300°C (dec.); IR (Nujol): 3370, 3310, 3180, 2450, 1640, 1560 cm^{-1}; UV (1% $NaHCO_3$ solution) λ_{max} 265 nm (ε, 28254); NMR (DMSO-d_6): δ 6.86 (1H, s, 7-H on pyridazine ring), 8.07 (2H, br-s, $-NH_2$).

Anal. Calcd. for $C_4H_4N_6S$: C, 28.54; H, 2.37; N, 49.94; S, 19.02.
Found: C, 28.35; H, 2.29; N, 49.73; S, 18.81.

11g; mp 185~187°C (dec.); IR (Nujol): 2480, 1675 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 262 nm (ε, 16048); NMR (DMSO-d_6): δ 3.48 (1H, br, –SH), 8.30 (1H, br, $-NH_2$), 8.55 (1H, s, 7-H on pyridazine ring), 8.64 (1H, br, $-NH_2$).

Anal. Calcd. for $C_5H_4N_6OS$: C, 30.60; H, 2.05; N, 42.84; S, 16.34.
Found: C, 30.65; H, 2.00; N, 42.63; S, 16.16.

7-Amino-3-[(tetrazolo[1,5-b]pyridazin-8-carboxy-6-yl)-thiomethyl]-3-cephem-4-carboxylic Acid (**12f**)

To a hot solution (40°C) of **11f** (1.97 g, 10 mmole) and $NaHCO_3$ (2.52 g, 30 mmole) in 0.1 M phosphate buffer (pH 6.4, 90 ml) 7-ACA (3.34 g, 12 mmole) was added portionwise. The mixture was stirred for 5 hours at 60°C, maintaining the pH between 6.8~7.2 by adding 5% $NaHCO_3$ or 3 N HCl if necessary.

The solution was treated with a small amount of charcoal and filtered. The filtrate was cooled in an ice-bath and adjusted to pH 2~3 with 23% HCl. The resulting precipitate was collected, the solid was suspended in a mixture of acetone - water (2: 1), stirred for 30 minutes, filtered, washed with water (15 ml) and acetone (15 ml) and dried *in vacuo* at 60°C to give 3.15 g (77%) of crude **12f**, which was used without further purification; mp 245°C (dec.); IR (Nujol): 1770 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 247 nm (ε, 21737), 320 (ε, 5649).

Anal. Calcd. for $C_{13}H_{11}N_7O_5S_2$: C, 38.13; H, 2.70; N, 23.94; S, 15.66.
Found: C, 38.45; H, 2.90; N, 23.65; S, 15.30.

By a similar procedure the derivatives **12** (**b**~**e**, **g**, **h**) were prepared and the data for each compound are as follows:

12b; mp 250°C (dec.); IR (KBr) 1800 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 242 nm (ε, 22114).

Anal. Calcd. for $C_{13}H_{13}N_7O_3S_2$: C, 41.15; H, 3.45; N, 25.84; S, 16.90.
Found: C, 40.91; H, 3.31; N, 25.61; S, 16.70.

12c; mp 250°C (dec.); IR (KBr) 1800 cm^{-1}; UV (1% $NaHCO_3$ solution) λ_{max} 262 nm (ε, 21874).

Anal. Calcd. for $C_{12}H_{12}N_8O_3S_2$: C, 37.88; H, 3.18; N, 29.45; S, 16.85.
Found: C, 37.51; H, 3.01; N, 29.22; S, 16.41.

12d; mp 260°C (dec.); IR (KBr) 1800 cm^{-1}; UV (1% NaOH solution) λ_{max} 269 nm (ε, 23587), 296 (ε, 16585).

Anal. Calcd. for $C_{13}H_{14}N_8O_3S_2$: C, 39.58; H, 3.57; N, 28.41; S, 16.25.
Found: C, 39.21; H, 3.31; N, 28.11; S, 15.93.

12e; mp 265°C (dec.); IR (KBr) 1805 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 270 nm (ε, 20299), 300 (ε, 18546).

Anal. Calcd. for $C_{14}H_{14}N_8O_5S_2$: C, 38.35; H, 3.21; N, 25.55; S, 14.62.
Found: C, 38.11; H, 3.01; N, 25.27; S, 14.31.

12g; mp 228~230°C (dec.); IR (Nujol): 1780 cm^{-1}; UV (1% $NaHCO_3$ solution) λ_{max} 249 nm (ε, 21679), 270s, 332 (ε, 4432).

Anal. Calcd. for $C_{13}H_{12}N_8O_4S_2$: C, 38.22; H, 2.96; N, 27.43; S, 15.70.
Found: C, 37.93; H, 2.93; N, 27.13; S, 15.45.

12h; mp 230°C (dec.); IR (KBr); 1800 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 264 nm (ε, 13429), 350 (ε, 4641).

Anal. Calcd. for $C_{12}H_{12}N_8O_3S_2$: C, 37.88; H, 3.18; N, 29.45; S, 16.85.
Found: C, 37.41; H, 3.01; N, 29.21; S, 16.39.

12a was prepared as reported in Reference[15].

Method A

7-[(*Z*)-β-Cyanovinylenethioacetamido]-3-[(tetrazolo[1,5-b]pyridazin-8-amino-6-yl)-thiomethyl]-3-cephem-4-carboxylic Acid (**19**)

To a stirred solution of (*Z*)-β-cyanovinylenethioacetic acid (0.72 g, 5 mmole) and triethylamine (0.70 ml) in dry acetone (40 ml), cooled to 0°C, pivaloylchloride (0.61 ml) dissolved in dry acetone (10 ml) was added dropwise. The mixture was stirred for 30 minutes at 0°C, then a solution of 7-amino-3-[(tetrazolo[1,5-b]pyridazin-8-amino-6-yl)-thiomethyl-3-cephem-4-carboxylic acid (1.90 g, 5 mmole) and triethylamine (0.7 ml) in 50% aqueous acetone (80 ml) was added dropwise, maintaining the temperature at about 0°C. After stirring for 1 hour at 0°C and 2 hours at room temperature the acetone was removed *in vacuo*. The residue was taken up with water and washed with ethyl acetate (discarded).

The aqueous phase was cooled, adjusted to pH 2 with 20% H_2SO_4 with stirring and extracted with ethyl acetate. The undissolved material was filtered off and discarded. The organic layer was separated, washed with aqueous NaCl solution, dried (Na_2SO_4) and evaporated to small volume *in vacuo*.

Dropwise addition of ethyl ether precipitated the product which was collected and dried *in vacuo* to give 1.85 g of **19**; mp 170~175°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20): Rf 0.56; UV (pH 7.4 phosphate buffer) λ_{max} 272 nm (ε, 31562); IR (KBr): 3300, 3150, 2210, 1770, 1630 cm^{-1}; NMR (DMSO-d_6): δ 3.68 (2H, q, 2-CH_2), 3.73 (2H, s, SCH_2CO), 4.31 (2H, q, 3-CH_2), 5.10 (1H, d, 6-H), 5.63 (1H, d-d, 7-H), 5.72 (1H, d, J=10 Hz, NC–CH=), 6.39 (1H, s, 7-H on pyridazine ring), 7.67 (1H, d, J=10 Hz, =CHS), 7.98 (2H, br-s, 8-NH_2 on pyridazine ring), 9.2 (1H, d, –CONH).

Anal. Calcd. for $C_{17}H_{15}N_9O_4S_3$: C, 40.38; H, 2.92; N, 25.00; S, 19.00.
Found: C, 40.10; H, 3.06; N, 24.70; S, 18.60.

Method B

7-[(*Z*)-β-Carboxyvinylenethioacetamido]-3-[(tetrazolo[1,5-b]pyridazin-8-amino-6-yl)-thiomethyl]-3-cephem-4-carboxylic Acid (**21**)

To a stirred solution of (*Z*)-β-*tert*-butoxycarbonylvinylenethioacetic acid (3.27 g, 15 mmole) in dry acetone (100 ml), cooled to −5°C were added triethylamine (2.11 ml) and 2 drops of *N*-methylmorpholine followed by a solution of pivaloyl chloride (1.83 ml) in dry acetone (20 ml). After stirring for 30 minutes at 5°C a solution of 7-amino-3-[(tetrazolo[1,5-b]pyridazin-8-amino-6-yl)-thiomethyl]-3-cephem-4-carboxylic acid (4.07 g, 10.7 mmole) in 50% aqueous acetone (150 ml) containing $NaHCO_3$ (0.9 g) and triethylamine (2.3 ml) was added dropwise. After stirring for 1 hour at 5°C and 2 hours at room temperature, the acetone was removed *in vacuo*, the undissolved material was filtered off and discarded. The aqueous phase was washed with ethyl acetate (discarded), adjusted to pH 2 with 20% H_2SO_4 under stirring and cooling. The solid was collected, washed with water and ethyl ether. The crude product was then stirred three times with a mixture of methanol - acetone (3: 1) (100 ml).

The solid was filtered off and discarded. The combined filtrates were evaporated *in vacuo* to give a solid product, which was then treated with ethyl acetate, filtered and washed with a small amount of acetone to give 4.35 g of 7-[(*Z*)-β-*tert*-butoxycarbonylvinylenethioacetamido]-3-[(tetrazolo[1,5-b]pyridazin-8-amino-6-yl)-thiomethyl]-3-cephem-4-carboxylic acid; mp 160°C (dec.); IR (KBr): 1760, 1650, 1575, 1370, 1160~1150 cm^{-1}.

Anal. Calcd. for $C_{21}H_{24}N_8O_6S_3$: C, 43.44; H, 4.17; N, 19.29; S, 16.56.
Found: C, 43.64; H, 4.32; N, 18.98; S, 16.34.

The above ester 2.9 g (5 mmole) was added to a stirred solution of trifluoroacetic acid (20 ml) and anisole (5 ml), cooled to −5°C. After stirring for 30 minutes at −5°C, the mixture was evaporated *in vacuo* below 40°C to remove trifluoroacetic acid. The resulting residue was taken up with benzene and evaporated again *in vacuo*. The residue was taken up with ethyl ether and collected. The solid was stirred with ethyl acetate (30 ml) for 1 hour and then filtered. The product was dissolved in 5% aqueous $NaHCO_3$ solution (75 ml), covered with ethyl acetate (500 ml) and adjusted to pH 4 with 20% H_2SO_4. A small amount of insoluble material was filtered off and discarded.

The organic layer was separated, washed with water, dried (Na_2SO_4) and evaporated *in vacuo* to small volume. After adding ethyl ether, a solid precipitated which was collected by filtration and dried *in vacuo* to give 1.53 g of **21**; mp 192~193°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 50: 20) Rf 0.54; IR (KBr): 1760, 1650, 1575 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 271 nm (ε, 29899); NMR (DMSO-d_6): δ 3.51 (2H, s, SCH_2CO), 3.58 (1H, d, 2-CH_2), 3.88 (1H, d, 2-CH_2), 4.13 (1H, d, 3-CH_2), 4.56 (1H, d, 3-CH_2), 5.13 (1H, d, 6-H), 5.71 (1H, d-d, 7-H), 5.85 (1H, d, J=10 Hz, HOOC–CH=), 6.36 (1H, s, 7-H on pyridazine ring), 7.42 (1H, d, J=10 Hz, =CHS), 7.95 (2H, br-s, 8-NH_2 on pyridazine ring), 9.14 (1H, d, –CONH).

Anal. Calcd. for $C_{17}H_{16}N_8O_6S_3$: C, 38.92; H, 3.07; N, 21.36; S, 18.33.
Found: C, 38.81; H, 3.25; N, 21.10; S, 17.99.

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