CEPHALOSPORINS IV

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NEW 7α-METHOXY-7β-VINYLENETHIOACETAMIDO CEPHALOSPORINS

ETTORE PERRONE, GIULIANO NANNINI*, FRANCO GIUDICI, DINO SEVERINO and PIER NICOLA GIRALDI

Department of Chemistry

GIUSEPPE MEINARDI and ANGELO CERIANI

Department of Biology
Farmitalia-Carlo Erba SpA, Research and Development,
Via C. Imbonati, 24, 20159 Milan, Italy

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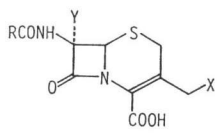
The synthesis and *in vitro* activity of 7α-methoxy-7β-vinylenethioacetamido cephalosporins with various substituents at the 3-position are described. These cephalosporins showed good activity against β-lactamase producing Gram-negative bacteria. 7α-Methoxy-7-[(*Z*)-β-cyanovinylenethioacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (**3**) was several times more active *in vitro* than cefoxitin and comparable to cefmetazole.

A few years ago we disclosed[1~3] a new class of alkylthioacetamido cephalosporins (**Ia**), among which 7-cyanomethylthioacetamido-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (K 10299) was the most active.

A significant increase in activity was achieved with a series of 7-vinylenethioacetamido cephalosporins (**Ib**)[4~7], which resulted several times more active than their saturated analogues (**Ia**). As an extension of our program, we decided to prepare their 7α-methoxy congeners (**Id**). The cephamycins (**Ic**) derived from **Ia** are interesting compounds; CS-1170[8] (cefmetazole), the 7α-methoxy derivative of our K 10299, is one of the best cephamycins currently available. As **Ib** constitute a definite improvement over **Ia**, the planned 7-vinylenethioacetamido cephamycins (**Id**) were expected to possess a still higher *in vitro* activity than their saturated congeners (**Ic**), coupled with the broadening of the antibacterial spectrum general for this class of compounds.



(I)

a $R = R_1CH_2SCH_2$, $Y = H$ **c** $R = R_1CH_2SCH_2$, $Y = OCH_3$
b $R = R_1CH{=}CHSCH_2$, $Y = H$ **d** $R = R_1CH{=}CHSCH_2$, $Y = OCH_3$

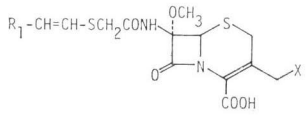
R_1 = CN, COOH
X = OAc, S-Het

In particular, the synthesis of compounds (**3**), (**4**) and (**6**) (Table 1), which are the 7α-methoxy derivatives of our most promising vinylenethioacetamido cephalosporins (respectively K 13101[4,5], K 13102[6,7] and K 13176[6,7]), was devised[9]. This paper describes the preparation of the new antibiotics and the result of our structure-activity studies.

Chemistry

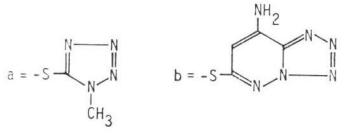
The side chain acids (R_1=CN, COOH) were already reported in a previous paper[4,5]. The syn-

Table 1. 7α-Methoxy-7β-(substituted vinylenethioacetamido) cephalosporins.



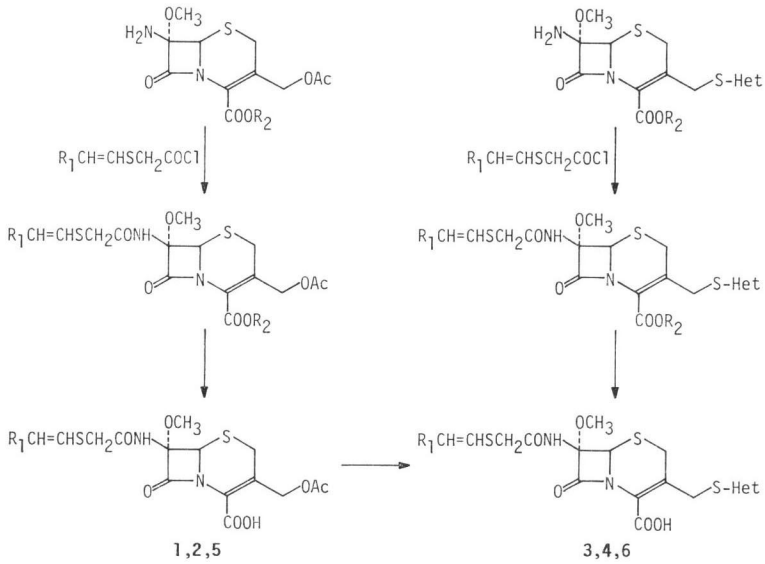
Compound	R_1	Configuration	X[a]	Method	IR (β-lactam) KBr, cm^{-1}	Formula[b]
1	NC	*E*	OAc	A	1780	$C_{16}H_{17}N_3O_7S_2$
2	NC	*Z*	OAc	A	1780	$C_{16}H_{17}N_3O_7S_2$
3	NC	*Z*	a	A	1780	$C_{16}H_{17}N_7O_5S_3$
4	NC	*Z*	b	C	1775	$C_{18}H_{17}N_9O_5S_3$
5	HOOC	*Z*	OAc	B	1775	$C_{16}H_{18}N_2O_9S_2$
6	HOOC	*Z*	b	C	1780	$C_{18}H_{18}N_8O_7S_3$

a)



b) All compounds were analysed for C, H, N, S. Analytical results are coincident with the calculated value within $\pm 1\%$ deviation.

Scheme 1.



thesis of 6-mercapto-8-amino-tetrazolo[1,5-b]pyridazine was also described[6,7]. The cephalosporins were prepared by coupling the unsaturated acids with the *tert*-butyl or the diphenyl-methylester of 7α-methoxy-7β-aminocephalosporanic acid or the *tert*-butylester of 7α-methoxy-7β-amino-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (see Scheme 1); the latter were obtained substantially according to the quinoidal imine method developed by YANAGISAWA *et al*[10]. Acylation was carried out by first converting the acid into the acyl chloride (**1**$\sim$**3**) (method A); the mixed anhydrides

Table 2. *In vitro* antibacterial activities of 7α-methoxycephalosporins.

Compound	MIC (μg/ml)[a]									
	S. a.	*S. a.*(R)	*E. c.*	*K. p.*	*S. t.*	*E. cl.*	*P. m.*	*P. v.*	*K. ae.*	*E. c.* t.
1	0.8	1.6	17.7	1.1	12.5	50	6.2	4	25	17.7
2	0.4	1.6	6.2	2.2	3.1	12.5	3.1	4	6.2	6.2
3	0.2	0.57	2.2	0.2	0.5	1.6	1.6	0.5	2.2	1.6
4	0.4	3.1	25	0.8	12.5	100	6.2	1.6	100	25
5	1.6	8.8	2.2	1.1	1.1	2.2	3.1	2.2	2.2	2.2
6	1.6	12.5	3.1	0.16	0.8	0.57	0.8	0.2	1.6	1.6
K 13101	0.025	0.14	0.14	0.1	0.07	0.4	0.4	>100	>100	6.2
K 13102	0.006	0.05	0.4	0.05	0.2	2.2	0.4	>100	>100	25
K 13176	0.05	0.57	0.28	0.05	0.1	0.05	0.05	50	>100	25
Cefazolin	0.05	0.57	1.6	0.8	1.6	3.1	6.2	>100	>100	12.5
Cefoxitin	0.8	4.4	8.8	2.2	3.1	17.8	6.2	3.1	6.2	8.8
Cefmetazole[b]	0.28	0.8	1.1	0.4	0.56	2.2	3.1	1.6	1.6	1.6

[a] Organisms included in this Table are: *S. a.*, *Staphylococcus aureus* Smith (penicillin G sensitive); *S. a.* (R), *Staphylococcus aureus* 39/2 (penicillin G resistant); *E. c.*, *Escherichia coli* G; *K. p.*, *Klebsiella pneumoniae* ATCC 10031; *S. t.*, *Salmonella typhi* Watson; *E. cl.*, *Enterobacter cloacae* 1321 E; *P. m.*, *Proteus mirabilis* ATCC 9921; *P. v.*, *Proteus vulgaris* X 20; *K. ae.*, *Klebsiella aerogenes* 1082 E; *E. c.* t., *Escherichia coli* TEM.

[b] Prepared in our laboratories.

previously employed[4~7] gave poor results. When a second carboxy group was present in the acylating acid (R_1=COOH) (**5**), this was protected as its *tert*-butyl ester, which was subsequently removed with trifluoroacetic acid-anisole (method B). **4** and **6** were obtained from **2** and **5** respectively by displacing the 3-acetoxy group with 8-aminotetrazolo[1,5-b]pyridazine thiol (method C).

The cephalosporins synthesized are listed in Table 1. The purity of the cephalosporins, established by NMR, TLC and analyses was greater than 90%.

Antimicrobial Activity

The minimum inhibitory concentrations (MIC) of this series of cephalosporins against 2 strains of Gram-positive and 8 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 reported in Table 2 are the geometric average of two determinations.

We compared the biological data of the 7α-OCH_3 cephalosporins synthesized with those of some 7α-hydrogen analogs (K 13101, K 13102, K 13176); the results were further compared with cefazolin, cefoxitin and cefmetazole (Table 2). The data show the following relationships between structure and activity:

1) In line with previous observations[11,12], the cephamycins (**3**, **4** and **6**) were less active than the corresponding 7α-hydrogen analogs (K 13101, K 13102 and K 13176) against bacteria which are usually sensitive to cephalosporins. On the other hand **3** and **6** showed marked activity against β-lactamase producing Gram-negative bacteria, while **4** showed higher activity than its 7α-hydrogen congener (K 13102) only against *Proteus vulgaris* X 20.

2) As expected[4~7] the (*E*)-isomer (**1**) was in general less active than its corresponding (*Z*)-isomer (**2**).

Table 3. *In vitro* activities of cephalosporins **3**, K 13101, cefoxitin and cefmetazole against clinically isolated Gram-negative bacteria.

Organisms	No. of strains tested	Compounds	Number of strains inhibited at concentration (μg/ml)									
			≤0.39	0.78	1.56	3.12	6.25	12.5	25	50	100	>100
Escherichia coli	20	**3**		1	11	3	2	3				
		K 13101	12	1	2			1	1	1		2
		Cefoxitin				1	13	2	2	2		
Klebsiella spp.	20	**3**		6	10	2	1	1				
		K 13101	6	1	2	2	4	3		2		
		Cefoxitin			1	11	7		1			
Proteus mirabilis	20	**3**		1	3	8	2	3	2	1		
		K 13101	3	6	2			2	1	1	3	2
		Cefoxitin				4	14	1		1		
Indole-positive *Proteus*	14	**3**		1	3	2	2	1			1	4
		K 13101							1	1	2	10
		Cefoxitin			2	3	2	2		1	3	1
		Cefmetazole		1	2	3	2	1			1	4

3) Displacement of the 3-acetoxy group (**2**) with 1-methyltetrazole thiol (**3**) enhanced the activity against Gram-positive and Gram-negative bacteria, including β-lactamase producing strains.

4) Displacement of the 3-acetoxy group (**2**, **5**) with 8-aminotetrazolo[1,5-b]pyridazine thiol (**4**, **6**) maintained the constant activity against Gram-positive bacteria, but produced different results against Gram-negative ones, depending on the side chain at 7-position. In compound containing a –CN group (**4**) we obtained in general a decrease of the activity, with the exceptions of *Klebsiella pneumoniae* ATCC 10031 and *Proteus vulgaris* X20. Conversely, in the derivative containing a –COOH group (**6**) the activity was enhanced, with the exception of *Escherichia coli* G.

The most interesting compound was **3**, which was 4~8 times more active than cefoxitin against Gram-positive bacteria and 2~10 times more active against Gram-negative ones including resistant strains. Cefmetazole and derivative **3** showed comparable activity against sensitive and β-lactamase producing Gram-negative bacteria.

3 was selected for further *in vitro* studies against clinical isolates of *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* (20 strains of each) in comparison with K 13101 and cefoxitin. The activity of **3** was also studied against indole-positive *Proteus* (14 strains) in comparison with K 13101, cefoxitin and cefmetazole (Table 3). Against *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* no great difference in activity was observed between **3** and its 7α-hydrogen analog (K 13101), but both were more active than cefoxitin. Against indole-positive *Proteus* **3** was more active than K 13101, but equivalent to cefoxitin and cefmetazole.

In contrast to our previous observations with 7α-hydrogen analogs[4,5], no improvement over the antibacterial activity of cefmetazole was therefore obtained by introducing a double bond in the 7-position side chain.

Experimental

Infrared spectra were recorded on a Perkin-Elmer spectrometer (model 125). The NMR spectra were determined on either a Perkin-Elmer R-24B (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 cor-

rected. Melting points of the cephalosporins are not accurately reproducible because of extensive decomposition.

(*Z*)-β-Cyanovinylenethioacetyl Chloride

To an ice-cold solution of (*Z*)-β-cyanovinylenethioacetic acid[4,5] (1.43 g, 10 mmole) in anhydrous ethyl ether (70 ml) PCl_5 (2.08 g) was added portionwise and the mixture was stirred for 2 hours at 5°C. The reaction mixture was then evaporated under reduced pressure below 40°C; the residue was taken up repeatedly with anhydrous benzene and evaporated under reduced pressure to remove all the $POCl_3$ formed during the reaction, yielding 1.61 g (100%) of the desired acyl chloride as a colorless oil, which was used immediately for the next step without further purification.

By a similar procedure (*E*)-β-cyanovinylenethioacetyl chloride was prepared starting from (*E*)-β-cyanovinylenethioacetic acid[4,5].

(*Z*)-β-*tert*-Butoxycarbonylvinylenethioacetyl Chloride

To a cold (−10°C) solution of (*Z*)-β-*tert*-butoxycarbonylvinylenethioacetic acid[4,5] (9.05 g, 41.4 mmole) in anhydrous ethyl ether (250 ml), PCl_5 (8.64 g) was added in a single portion. The resulting suspension was stirred for 5 minutes at −10°C and then for 2 hours at 2~5°C. The resulting colorless solution was evaporated under reduced pressure; the residue was taken up repeatedly with anhydrous benzene and evaporated under reduced pressure to remove all the $POCl_3$ formed during the reaction, giving 9.8 g (100%) of the desired acyl chloride as a white crystalline mass, which was used immediately for the next step without further purification.

Method A

7α-Methoxy-7-[(*Z*)-β-cyanovinylenethioacetamido]cephalosporanic Acid (**2**)

To an ice-cold solution of the *tert*-butyl ester of 7β-amino-7α-methoxycephalosporanic acid (4.5 g, 12.55 mmole) in anhydrous 1,2-dichloroethane (80 ml), a solution of *N*,*N*-diethylaniline (1.61 ml) and (*Z*)-β-cyanovinylenethioacetyl chloride (1.61 g, 10 mmole) in anhydrous 1,2-dichloroethane (30 ml) was added. After stirring for 30 minutes at 0°C, the reaction mixture was diluted with ethyl acetate and sequentially washed with 3% aqueous $KHSO_4$ solution, water, 5% aqueous $NaHCO_3$ solution and water.

The organic layer was separated, dried (Na_2SO_4) and evaporated *in vacuo*. The resulting oil was chromatographed (silica gel, ethyl acetate - cyclohexane, 2: 1). Evaporation to dryness under reduced pressure yielded 2.4 g (50%) of the *tert*-butyl ester; IR (KBr) 2200, 1780, 1685 cm^{-1}; NMR ($CDCl_3$) δ 1.56 (9H, s, –COO-*t*Bu), 2.08 (3H, s, $-OCOCH_3$), 3.25 (1H, d, 2-CH_2), 3.55 (3H, s, 7-OCH_3), 3.57 (1H, d, 2-CH_2), 3.63 (2H, s, SCH_2CO), 4.79 (1H, d, 3-CH_2), 5.05 (1H, d, 3-CH_2), 5.07 (1H, s, 6-H), 5.43 (1H, d, J=10 Hz, NC–CH=), 7.44 (1H, d, J=10 Hz, =CHS), 7.92 (1H, s, –CONH).

A solution of the above ester (3.0 g) in anisole (20 ml), prepared by first dissolving the reagents in dichloromethane and then evaporating the solvent, was treated at 0°C with trifluoroacetic acid (40 ml). After 1 hour at room temperature the mixture was evaporated under reduced pressure to remove the anisole and the trifluoroacetic acid. The residue was taken up with ethyl acetate (20 ml), treated with activated charcoal, filtered, and dicyclohexylamine (0.65 ml) was added. The solution was cooled and the dicyclohexylamine salt was filtered, washed with ethyl acetate and then with ethyl ether. The solid was suspended in water, layered with ethyl acetate and adjusted to pH 2 with 20% H_2SO_4. After stirring for 15 minutes, the organic phase was separated, washed with water, dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The residue was taken up with ethyl ether to give 1.32 g (50%) of **2**; m.p. 105~110°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 20: 20), Rf 0.43; IR (KBr) 2200, 1780, 1680, 1570 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 272 nm (ε, 16371); NMR (DMSO-d_6) δ 2.02 (3H, s, $OCOCH_3$), 3.27 (1H, d, 2-CH_2), 3.4 (3H, s, 7-OCH_3), 3.62 (1H, d, 2-CH_2), 3.76 (2H, s, SCH_2CO), 4.67 (1H, d, 3-CH_2), 4.98 (1H, d, 3-CH_2), 5.16 (1H, s, 6-H), 5.43 (1H, d, J=10 Hz, NC–CH=), 7.44 (1H, d, J=10 Hz, =CHS), 9.52 (1H, s, –CONH).

Anal. Calcd. for $C_{16}H_{17}N_3O_7S_2$: C 44.95, H 4.01, N 9.83, S 15.00.
Found C 44.57, H 4.26, N 9.53, S 14.48.

By a similar procedure **1** and **3** were prepared and the data for each compound are as follows:

1; mp 95~100°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 20: 20), Rf 0.35; IR (KBr) 2200, 1780, 1730 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 272 nm

(ε, 15870); NMR (DMSO-d_6) δ 2.02 (3H, s, $OCOCH_3$), 3.30 (1H, d, 2-CH_2), 3.4 (3H, s, 7-OCH_3), 3.62 (1H d, 2-CH_2), 3.76 (2H, s, SCH_2CO), 4.67 (1H, d, 3-CH_2), 4.98 (1H, d, 3-CH_2), 5.16 (1H, s, 6-H), 5.67 (1H, d, J=16 Hz, NC–CH=), 7.85 (1H, d, J=16 Hz, =CHS), 9.58 (1H, s, –CONH).

Anal. Calcd. for $C_{16}H_{17}N_3O_7S_2$: C 44.95, H 4.01, N 9.83, S 15.00.
Found C 44.57, H 4.11, N 9.41, S 14.23.

3; mp 118~120°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20), Rf 0.67; IR (KBr) 2200, 1780, 1630, 1520 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 275 nm (ε, 18713); NMR (DMSO-d_6) δ 3.4 (3H, s, 7-OCH_3), 3.6 (2H, q, 2-CH_2), 3.78 (2H, s, SCH_2CO), 3.94 (3H, s, $-NCH_3$), 4.36 (2H, q, 3-CH_2), 5.00 (1H, s, 6-H), 5.74 (1H, d, J=10 Hz, NC–CH=), 7.68 (1H, d, J=10 Hz, =CHS), 9.56 (1H, s, –CONH).

Anal. Calcd. for $C_{13}H_{17}N_7O_5S_3$: C 39.74, H 3.54, N 20.27, S 19.89.
Found C 39.97, H 3.71, N 19.91, S 19.41.

Method B

7α-Methoxy-7-[(*Z*)-β-carboxyvinylenethioacetamido]cephalosporanic Acid (**5**)

To a solution of the diphenylmethyl ester of 7β-amino-7α-methoxycephalosporanic acid (15.6 g, 33 mmole), cooled to −10°C, in anhydrous 1,2-dichloroethane (250 ml), *N*,*N*-diethylaniline (6.62 ml) was added followed immediately by a solution of (*Z*)-β-*tert*-butoxycarbonylvinylenethioacetyl chloride (6.15 g, 26 mmole) in anhydrous 1,2-dichloroethane (90 ml). After stirring for 20 minutes at −5°C and then for 1 hour at room temperature, the solvent was removed under reduced pressure. The residue was taken up with ethyl acetate, the solution was washed with 3% aqueous $KHSO_4$ solution, water, 5% aqueous $NaHCO_3$ solution and water. The organic phase was separated, dried (Na_2SO_4) and chromatographed (silica gel, ethyl ether). The fractions containing the pure product were combined, concentrated until incipient separation of an oil and poured, with stirring, into 4 volumes of cyclohexane to give 17.19 g of the double ester, as a pale yellow powder.

The double ester thus obtained was added portionwise under stirring to a mixture of trifluoroacetic acid (150 ml) and anisole (25 ml), cooled to 0°C. After 30 minutes at 0°C, the trifluoroacetic acid was evaporated under reduced pressure without heating and the residue was triturated with ethyl ether. The precipitate was filtered, washed with ethyl ether and immediately dried over P_2O_5 to yield 10.05 g (87.6%) of **5**; mp 120~122°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 20: 30), Rf 0.38; IR (KBr) 1775, 1730, 1680 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 267 nm (ε, 14760); NMR (DMSO-d_6) 2.02 (3H, s, $OCOCH_3$), 3.41 (3H, s, 7-OCH_3), 3.44 (1H, d, 2-CH_2), 3.49 (2H, s, SCH_2CO), 3.65 (1H, d, 2-CH_2), 4.69 (1H, d, 3-CH_2), 5.03 (1H, d, 3-CH_2), 5.12 (1H, s, 6-H), 5.84 (1H, d, J=10 Hz, HOOC–CH=), 7.4 (1H, d, J=10 Hz, =CH–S), 9.58 (1H, br-s, –CONH).

Anal. Calcd. for $C_{16}H_{15}N_2O_9S_2$: C 43.04, H 4.06, N 6.27, S 14.36.
Found C 43.15, H 4.16, N 6.12, S 14.21.

Method C

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

To a solution of **5** (3.12 g, 6.98 mmole) and $NaHCO_3$ (1.765 g) in water (40 ml) 6-mercapto-8-aminotetrazolo[1,5-b]pyridazine (1.175 g, 6.98 mmole) was added and the pH corrected to 4.7 by adding $NaHCO_3$ or $KHSO_4$ if necessary. The reaction vessel was then placed in a thermostatic bath at 100~105°C. After 45 minutes the reaction was stopped by adding crushed ice to the mixture. The mixture was then salted with NaCl, extracted with ethyl acetate (150 ml) and acidified with 20% H_2SO_4. The organic phase was separated and the aqueous phase was extracted twice (2×50 ml) with ethyl acetate.

The combined organic phases were concentrated to small volume; addition of ethyl ether precipitated 1.95 g of crude product. Purification of this material could be easily effected through its double diphenylmethyl ester. Thus diphenyldiazomethane (4.04 g) in acetonitrile (140 ml) and dioxane (20 ml) was added, the suspension stirred for 30 minutes at room temperature, and the excess reagent decomposed with acetic acid. The mixture was evaporated to dryness under reduced pressure and the resulting material was fractionated by silica gel chromatography (ethyl acetate - cyclohexane, 2: 1) to give, after trituration with ethyl ether and filtration, 2.6 g of the pure double diphenylmethyl ester. This ester was

then added, in small portions, to a solution of trifluoroacetic acid (25 ml) and anisole (3 ml) at $-10°C$. After stirring for 10 minutes, the trifluoroacetic acid was removed under reduced pressure and the residue was triturated with ethyl ether (60 ml), filtered and washed with ethyl ether to afford 1.35 g (35%) of **6**; mp 180°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20), Rf 0.47; IR (KBr) 1780, 1710~1680, 1630, 1570 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 267 nm (ε, 28530); NMR (DMSO-d_6) δ 3.41 (3H, s, 7-OCH_3), 3.48 (1H, d, 2-CH_2), 3.55 (2H, s, -SCH_2CO), 3.80 (1H, d, 2-CH_2), 4.07 (1H, d, 3-CH_2), 4.53 (1H, d, 3-CH_2), 5.14 (1H, s, 6-H), 5.85 (1H, d, J=10 Hz, HOOC-CH=), 6.35 (1H, s, 7-H on pyridazine ring), 7.4 (1H, d, J=10 Hz, =CHS), 7.93 (2H, br-s, 8-NH_2 on pyridazine ring), 9.52 (1H, br-s, -CONH).

Anal. Calcd. for $C_{18}H_{18}N_8O_7S_3$: C 38.98, H 3.27, N 20.20, S 17.34.
Found C 39.10, H 3.29, N 20.05, S 17.23.

By a similar procedure **4** was prepared and the data are as follows:

mp 130~135°C (dec.); TLC on silica gel gave a single spot with chloroform - methanol - formic acid (160: 40: 20), Rf 0.55, IR (KBr) 3200~3550, 2210, 1775, 1680, 1640, 1570 cm^{-1}; UV (pH 7.4 phosphate buffer) λ_{max} 266 nm (ε, 29672); NMR (DMSO-d_6) δ 3.44 (3H, s, 7-OCH_3), 3.76 (2H, d, 2-CH_2), 3.50 (2H, s, -SCH_2CO), 4.11 (1H, d, 3-CH_2), 4.54 (1H, d, 3-CH_2), 5.16 (1H, s, 6-H), 5.73 (1H, d, J= 10 Hz, NC-CH=), 6.36 (1H, s, 7-H on pyridazine ring), 7.69 (1H, d, J=10 Hz, =CHS), 7.94 (2H, br-s, 8-NH_2 on pyridazine ring), 9.58 (1H, br-s, -CONH).

Anal. Calcd. for $C_{18}H_{17}N_9O_5S_3$: C 40.36, H 3.20, N 23.53, S 17.96.
Found C 39.70, H 3.29, N 23.31, S 17.35.

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