3-ACYLTHIOMETHYL CEPHALOSPORINS

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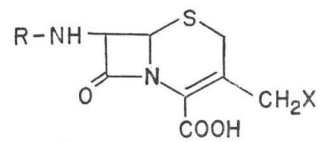
A series of new derivatives of 7-aminocephalosporanic acid in which the acetoxymethyl function at C_3 has been replaced with a heteroaromatic carbonylthiomethyl moiety and the 7-amino group has been acylated with D-phenylglycine has been prepared. Many of these derivatives were well absorbed following oral administration to mice.

Molecular modification of the natural product cephalosporin C (**1**) has resulted in the application to human chemotherapy of several cephalosporin antibiotics. The orally-absorbed antibiotics cephaloglycin[1] (**2**) and cephalexin[2] (**3**) have in common a D-phenylglycyl substituent attached to the amino group at C_7 of the cephem nucleus.[3] This same substituent has been associated with oral absorption in mice also in the 3-azidomethyl derivative[4] (**4**) and the 3-methoxymethyl derivative[5] (**5**). We have prepared a series of compounds (**6**) from 7-aminocephalosporanic acid (7-ACA) (**7**) in which the acetoxy function has been replaced by a heteroaromatic thiolcarbonyl moiety and the 7-amino group has been acylated with D-phenylglycine. Many of these new compounds were well absorbed following oral administration to mice.

Chemistry

For this series, we required heteroaromatic thiolcarboxylic acids of which surprisingly few have been described in the literature. The method of NOBLE and TARBELL[6] for the preparation of thiobenzoic acid, *i.e.* the reaction of a carboxylic acid chloride with sodium sulfhydrate in 90 % aqueous ethanol, has been applied to a wide variety of heteroaromatic carboxylic acids to obtain the corresponding thiolcarboxylic acids. These products could often be isolated and characterized as the free thiol acids, but in some cases it was preferable to isolate them as their dicyclohexylamine salts (in such cases the thiol acid was regenerated before reaction with 7-ACA). Heterocyclic carboxylic acids (all of which have been reported in the literature) containing 1, 2 or 3 heteroatoms in a 5-membered ring were in general successfully employed, but extensive decomposition occurred when the reaction was applied to the acid chlorides of 5-methylisoxazole-3-carboxylic acid and 5-nitrofuran-2-carboxylic acid. The thiolcarboxylic acids reacted with 7-ACA (**7**) in aqueous solution at pH 6~6.4 and 50~55°C by nucleophilic



	R	X
1	D-$HOOCCH(NH_2)(CH_2)_3CO$	$OCOCH_3$
2	D-$C_6H_5CH(NH_2)CO$	$OCOCH_3$
3	D-$C_6H_5CH(NH_2)CO$	H
4	D-$C_6H_5CH(NH_2)CO$	N_3
5	D-$C_6H_5CH(NH_2)CO$	OCH_3
6	D-$C_6H_5CH(NH_2)CO$	$SCOR^1$
7	H	$OCOCH_3$
8	H	$SCOR^1$

Table 1. The antibacterial activity (*in vitro*) of

No.	Compound (6), R^1=	%[b] purity	*Diplococcus pneumoniae* A-9585	*Streptococcus pyogenes* A-9604	*Staphylococcus aureus* Smith A-9537	*Staphylococcus aureus* Smith +50% serum
1	2-Furyl	85	.02	.02	.16	> 1.3
2	2-Thienyl	80	.16	.16	.16	> 5
3	3-Thienyl	90	1.3	1.3	. 3	>63
4	2-Pyrrolyl	90	.08	.08	>2. 5	16
5	1-Methyl-2-pyrrolyl	90	.04	.08	.16	32
6	3-Isothiazolyl	95	.02	.02	. 3	32
7	4-Isothiazolyl	95	.02	.04	.08	32
8	5-Isothiazolyl	95	.16	.16	. 3	32
9	3-Pyrazolyl	90	.04	.04	. 3	4
10	4-Pyrazolyl	85	.16	.08	. 6	32
11	2-Methyl-4-oxazolyl	95	.04	.04	. 6	16
12	3-Methyl-5-isoxazolyl	90	2.5	2.5	. 6	63
13	1-Methyl-3-pyrazolyl	85	.01	.02	. 3	32
14	1-Methyl-4-pyrazolyl	85	.16	.16	. 3	>63
15	1-Methyl-5-pyrazolyl	90	.08	.08	. 3	63
16	4-Methyl-3-pyrazolyl	80	.01	.01	. 3	32
17	5-Chloro-1-methyl-3-pyrazolyl	85	. 3	1. 3	. 6	>63
18	3,5-Dimethyl-4-isoxazolyl	95	.04	.04	. 3	>63
19	1,3,4-Triazol-2-yl	80	.16	.16	1. 3	>63
20	1,2,5-Triazol-3-yl	85	.08	.08	. 6	63
21	1,2,3-Thiadiazol-4-yl	90	.08	.08	. 3	32
22	1,2,3-Thiadiazol-5-yl	90	. 3	. 3	. 6	16
23	1,2,5-Thiadiazol-3-yl	85	.16	. 3	. 3	32
24	1-Methyl-1.2,3-triazol-4-yl	85	.016	.016	. 6	4
25	5-Methyl-1,2,3-thiadiazol-4-yl	90	.02	.02	. 3	32
26	4-Chloro-1,2,5-thiadiazol-3-yl	85	. 3	. 3	. 3	32
	Cephaloglycin		.06	.06	. 6	2. 5
	Cephalexin		.16	.16	1. 3	2. 5

[a]The MIC values are in μg/ml and were determined by a 2-fold serial dilution assay in Difco
[b]Estimated from nmr spectra; the contaminant was mainly D-phenylglycine.

displacement by sulfur of the acetoxy group to provide derivatives (**8**). These were characterized by their ir spectra which showed absorptions at 1805~1810 cm.$^{-1}$ (due to the β-lactam carbonyl) and at 1650~1660 cm.$^{-1}$ (due to the -S-C=O group), and by their nmr spectra which included an AB quartet near 4.2 ppm (from TMS) due to the exomethylene protons at C_3, and which showed no contamination by 7-ACA. These new derivatives of 7-ACA were extremely insoluble in both aqueous and non-aqueous solvents. They were therefore routinely silylated which allowed acylation with D-phenylglycyl chloride hydrochloride to be effected in a solvent such as methylene chloride. Aqueous hydrolysis of the acylated intermediate then provided the new cephalosporins (**6**). Some of these derivatives were non-crystalline, so the purity of each compound was estimated from the nmr spectrum and is included in Table 1.

Biological Results

The minimum inhibitory concentrations (MIC's) of the new cephalosporins against a variety

3-acylthiomethyl-cephalosphorins (6)

Staphylococcus aureus BX 1633-2 A-9606	*Salmonella enteritidis* A-9531	*Escherichia coli* Juhl A-15119	*Escherichia coli* A-9675	*Klebsiella pneumoniae* A-9977	*Klebsiella pneumoniae* A-15130	*Proteus mirabilis* A-9900	*Proteus mirabilis* A-15153
. 3	.25	2	4	1	16	1	16
. 5	.25	8	16	8	32	4	32
. 6	. 6	8	16	8	32	16	63
>2. 5	>2. 5	8	16	8	16	4	125
. 6	2. 5	32	32	16	32	16	125
. 3	.04	2	4	2	16	2	63
. 3	.08	8	16	8	16	8	63
. 6	.25	8	16	4	16	8	16
. 6	.08	1	4	1	8	1	32
2. 5	. 6	16	32	4	32	4	125
1. 3	.25	4	16	4	16	2	63
1. 3	.25	2	8	2	8	2	63
. 6	.16	4	8	4	16	2	63
1. 3	. 3	4	16	4	63	4	63
1. 3	1. 3	63	63	32	>125	63	125
. 6	1. 3	32	32	16	125	16	63
1. 3	. 5	16	32	4	125	16	32
. 6	16	>125	>125	125	>125	125	>125
2. 5	. 3	4	16	1	16	1	63
. 6	.08	2	8	.25	4	.25	63
. 3	.08	1	2	. 5	4	1	16
1. 3	. 3	8	32	4	32	4	32
. 6	.16	4	16	4	16	2	63
1. 3	.08	.3	8	.3	4	.6	32
. 6	. 3	16	16	16	63	16	125
. 6	. 6	32	32	16	63	4	63
1. 3	. 3	1	4	. 6	2	. 6	63
2	2	4	. 8	4	8	4	>125

nutrient broth by the method of PURSIANO *et al.*[7]

of gram-positive and gram-negative bacteria are compared with those of cephaloglycin and cephalexin in Table 1. The blood levels obtained following administration of an oral dose of 100 mg/kg to mice are recorded in Table 2. From the *in vitro* data it can be seen that over half of the compounds listed showed activity against the penicillin sensitive gram-positive organisms comparable to that of cephaloglycin. Against the β-lactamase-producing *Staphylococcus aureus* BX 1633-2 most of the new cephalosporins were more active than cephalexin and several were 2~4 fold more active than cephaloglycin. The MIC's for all of the compounds listed were strikingly high when measured in the presence of human serum. In an independent, quantitative experiment, it was found that the new compounds were bound to human serum to the extent of 80~99 %. Against the gram-negative microorganisms tested, only compounds **1**, **9**, **20**, **21** and **24** were comparable in their activity with cephaloglycin. None of the new cephalosporins gave MIC's of less than 125 μg/ml when tested against *Pseudomonas aeruginosa* A9843A or *Serratia marcescens* A20019. Structure-activity relationships in this series

Table 2. Oral mouse blood levels* of 3-acylthiomethyl-cephalosporins (6)

No.	Compound (6), R^1=	Minutes after administration				No. of experiments
		30	60	120	210	
1	2-Furyl	4.0	7.4	7.3	2.3	2
2	2-Thienyl	<2.2	<2.2	<2.2	<2.2	1
3	3-Thienyl	<2.2	<2.2	<2.2	<2.2	1
4	2-Pyrrolyl	0.5	0.6	0.5	0.2	1
5	1-Methyl-2-pyrrolyl	<0.7	1.0	<0.7	<0.7	1
6	3-Isothiazolyl	18.8	16.2	6.0	<0.9	2
7	4-Isothiazolyl	6.1	10.5	6.0	2.3	1
8	5-Isothiazolyl	23.1	14.8	4.7	<2.4	3
9	3-Pyrazolyl	0.3	0.8	0.6	<0.2	2
10	4-Pyrazolyl	0.7	0.8	0.6	<0.3	1
11	2-Methyl-4-oxazolyl	6.6	10.6	8.2	3.8	2
12	3-Methyl-5-isoxazolyl	33.9	27.1	8.3	2.1	4
13	1-Methyl-3-pyrazolyl	1.2	1.6	1.2	<1.1	1
14	1-Methyl-4-pyrazolyl	0.5	0.5	0.4	<0.3	1
15	1-Methyl-5-pyrazolyl	<0.7	1.9	1.4	<0.7	1
16	4-Methyl-3-pyrazolyl	0.3	0.3	<0.3	<0.3	1
17	5-Chloro-1-methyl-3-pyrazolyl	2.2	4.1	5.4	2.6	1
18	3,5-Dimethyl-4-isoxazolyl	<1.2	<1.2	<1.2	<1.2	1
19	1,3,4-Triazol-2-yl	6.5	8.0	3.8	<3.6	1
20	1,2,5-Triazol-3-yl	2.7	3.1	1.7	0.4	1
21	1,2,3-Thiadiazol-4-yl	17.7	11.8	3.8	0.8	1
22	1,2,3-Thiadiazol-5-yl	26.6	18.2	5.5	<1.6	3
23	1,2,5-Thiadiazol-3-yl	18.9	14.8	5.0	2.7	1
24	1-Methyl-1,2,3-triazol-4-yl	11.1	16.6	7.0	1.3	4
25	5-Methyl-1,2,3-thiadiazol-4-yl	5.2	8.8	5.0	<1.6	2
26	4-Chloro-1,2,5-thiadiazol-3-yl	14.3	12.5	8.5	2.5	1
	Cephalexin	44.6	23.4	6.1	1.7	10
	Cephalogycin	6.1	5.4	2.6	0.6	1

* Compounds administered at a dosage of 100 mg/kg. Results are expressed in μg/ml and are the average of 8 mice per experiment. The assay organism was *Sarcina lutea*.

are tenuous. In the series of unsubstituted isothiazoles, **6**~**8**, the most active compound *in vitro* (**6**) had the carbonylthiomethyl substituent adjacent to the nitrogen atom of the ring. This was true also of the pyrazole derivatives **9**, **10**, **13**, and **14**, and the 1,2,3-thiadiazole derivatives **21** and **22**. In general, introduction of a methyl substituent (compare **13**, **16** with **9**; **25** with **21**; **5** with **4**) or a chlorine substituent (compare **17** with **13**; **26** with **23**) gave less active compounds, especially with regard to gram-negative activity. The compound (**18**) with the least activity against gram-negative microorganisms was the only one prepared which contained 2 methyl substituents in the heterocyclic ring. From the *in vivo* data in Table 2 it is apparent that the new compounds gave peak blood levels at the 0.5~1.0 hour time period. About half of the new cephalosporins showed higher peak blood levels than did cephaloglycin, but none of these compounds gave a peak blood level as great as that of cephalexin. In a few cases (**1**, **11**, **12** and **26**) the blood levels at the 120 and 210 minutes time intervals were slightly higher than the values for cephalexin. In general, the compounds containing only 1 heteroatom in the

ring gave low blood levels as did compounds in which all the ring heteroatoms were nitrogen. Introduction of oxygen or sulfur into the ring tended to increase the blood levels.

Experimental Section

Since the preparations of the thiolcarboxylic acids, 7-ACA derivatives and final products were carried out in essentially the same manner for all the compounds listed in the Tables, the experimental details for only one sequence are included here. Melting points were recorded in capillary tubes on a Mel-Temp apparatus. Ir spectra were recorded on a Beckman IR-5 Spectrophotometer and nmr spectra were run at 60 MHz with a Perkin-Elmer R12B spectrophotometer.

Isothiazole-5-thiolcarboxylic acid

A mixture of 15 g of isothiazole-5-carboxylic acid and 75 ml of thionyl chloride was heated under reflux for 12 hours. The excess reagent was removed under reduced pressure and the residue was distilled at 41~43°/1.5 mm to provide 14.5 g (.0985 mole) of the acid chloride. This was added dropwise to a stirred and cooled solution of 22.0 g (.20 mole) of hydrated sodium sulfhydrate in 225 ml of ethanol and 25 ml of water at such a rate as to keep the temperature of the mixture at 10~15°C. After the addition was completed, the reaction mixture was stirred for 40 minutes at 5~10°C. Most of the ethanol was removed under reduced pressure and the residue was dissolved in 130 ml of water. The pH of the solution was lowered to 2.8 by addition of 6 N hydrochloric acid and was maintained there while the mixture was extracted with 5×100 ml portions of ethyl acetate. The extracts were combined, washed with ice-water, dried over magnesium sulfate and evaporated to dryness to give 11.7 g (82 %) of yellow solid, mp 90~91°C (dec). An analytical sample was prepared by sublimation at 65°/0.05 mm which provided yellow crystals of mp 93~94°C (dec).

Anal. calcd. for $C_4H_3NOS_2$: C, 33.09; H, 2.08; N, 9.65; S, 44.17
Found: C, 32.83; H, 2.01; N, 9.78; S, 44.39

2-Methyloxazole-4-thiolcarboxylic acid dicyclohexylamine salt

The acid chloride was reacted with sodium sulfhydrate as above, but on evaporation of the ethyl acetate extracts an oily residue was obtained. This was dissolved in ether and treated dropwise with an equivalent of dicyclohexylamine. The crystalline salt was collected by filtration and dried *in vacuo*; mp 161°C (dec).

Anal. calcd. for $C_{17}H_{28}N_2O_2S$: C, 62.92; H, 8.68; N, 8.64; S, 9.88
Found: C, 62.75; H, 8.86; N, 8.48; S, 9.97

7-Amino-3(isothiazol-5-ylcarbonylthiomethyl)-3-cephem-4-carboxylic acid

To a stirred solution of 18.0 g (.066 mole) of 7-aminocephalosporanic acid and 11.1 g (.132 mole) of sodium bicarbonate in 320 ml of aqueous phosphate (buffered at pH 6.4) was added 9.6 g (.066 mole) of isothiazole-5-thiocarboxylic acid. The mixture was stirred in a nitrogen atmosphere at 50°C for 5 hours and was then cooled to 20°C. The precipitated solid was collected by filtration, washed with water and acetone and dried *in vacuo* over phosphorus pentoxide to provide 8.5 g of crystalline solid, mp 208~210°C (dec).

Anal. calcd. for $C_{12}H_{11}N_3O_4S_3 \cdot 0.5\ H_2O$: C, 39.33; H, 3.30; N, 11.47; H_2O, 2.46
Found: C, 39.49; H, 3.08; N, 11.69; H_2O, 1.61

A second crop of 2.8 g was obtained by lowering the pH of the filtrate to 6.2 with 6 N hydrochloric acid and collecting the solid as described above; total yield 11.3 g, 47 %.

7-[D(−)-α-Aminophenylacetamido]-3(isothiazol-5-ylcarbonylthiomethyl)-3-cephem-4-carboxylic acid

To a stirred slurry of 5.49 g (.015 mole) of 7-amino-3 (isothiazol-5-ylcarbonyl-thiomethyl)-3-cephem-4-carboxylic acid hemihydrate in 150 ml of dry methylene chloride were added in succesion 2.73 g (.027 mole) of triethylamine, 3.99 g (.033 mole) of N, N-dimethylaniline and 4.91 g (.045 mole) of trimethylchlorosilane. The solution was heated under reflux for 0.5 hour and

was then cooled to 4°C and treated with 3.40 g (.0165 mole) of D-phenylglycyl chloride hydrochloride. The slurry was stirred for 1 hour at 4~6°C and for 0.75 hour without external cooling. Cold water (80 ml) was added with stirring and the emulsion which formed was added to an excess of ethyl acetate. A solid was removed by filtration and the aqueous phase was separated from the filtrate and layered with fresh ethyl acetate. The pH was adjusted to 4.0 with 2N sodium hydroxide and the product which precipitated was collected by filtration, washed with water and ethyl acetate and dried *in vacuo* over phosphorus pentoxide to yield 3.8 g (52%), mp 168~170°C (dec). Infrared spectrum (KBr disc) had absorption maxima (cm^{-1}) at 1780 (β-lactam carbonyl), 1690 (amide carbonyl), 1645 (thiolester carbonyl), 1605 and 1395 (carboxylate), and 710 (phenyl). The nmr spectrum of a solution of the cephalosporin derivative in d_6-dimethylsulfoxide, deuterium oxide (1:2) and deuterium chloride (trace) showed absorptions [ppm (δ) from tetramethylsilane] which were assigned as follows: doublets at 8.61 (1H) and 7.82 (1H) for the isothiazole ring protons, singlet (5H) at 7.50 due to the benzene ring protons, doublets at 5.76 (1H) and 5.08 (1H) due to the β-lactam ring protons, singlet (1H) at 5.30 for the benzylic proton, AB pattern (2H) centered at 4.18 due to the exocyclic methylene protons, AB pattern (2H) centered at 3.50 due to the protons at C_2 of the dihydrothiazine ring.

Anal. calcd. for $C_{20}H_{18}N_5O_5S_3 \cdot H_2O$: C, 47.23; H, 3.96; N, 11.02
Found: C, 47.43; H, 4.21; N, 11.33

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