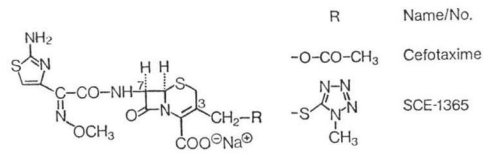
Ro 13-9904/001, A NOVEL POTENT AND LONG-ACTING PARENTERAL CEPHALOSPORIN*

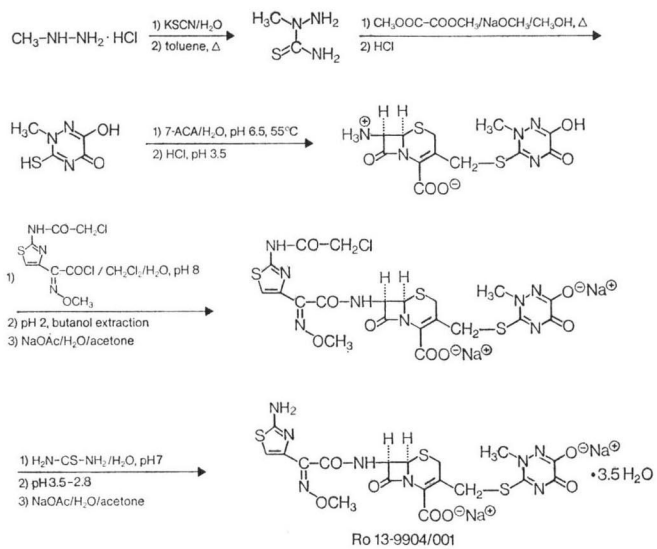
Sir:

In recent years a number of highly active new cephalosporins have been reported. While conducting a research program on β-lactams we became interested in the effects of structural modification of the 3-heterocyclic-thiomethyl moiety on the biological activities and especially the pharmacokinetic parameters in the series of 7-{2-(2-amino-4-thiazolyl)-2-[(*Z*)-methoxyimino] acetamido} cephalosporins. Compounds belonging to this series, such as cefotaxime[1,2] and

Fig. 1. Structure of cefotaxime and SCE-1365.



Scheme 1. Synthesis of Ro 13-9904/001.



Ro 13-9904/001

Table 1. Physico-chemical and analytical data of Ro 13-9904/001.

1)	Empirical formula: $C_{18}H_{16}N_8O_7S_3Na_2 \cdot 3.5\ H_2O$				
2)	Molecular weight: 661.59				
3)	Elemental analysis:				
	Calcd.	C 32.68	H 3.50	N 16.94	S 14.54	H_2O 9.53
	Found	C 32.89	H 3.46	N 16.96	S 14.54	H_2O 9.50
4)	Form: White crystalline powder				
5)	Melting point: >155°C (dec.)				
6)	Specific rotation: $[\alpha]_D^{25} -165°$ (*c* 1, water) (calcd. for water-free substance)				
7)	Rf (silicagel F_{254}-precoated plate "Merck"; methanol-water, 9: 1): 0.73				
8)	Solubility in water: *ca.* 40 g/100 ml (25°C)				
9)	pH of 20% aqueous solution: 7.1				
10)	pKa values: *ca.* 3 (COOH), 3.2 ($NH_3^{\oplus}$), 4.1 (enolic OH)				
11)	IR spectrum (KBr pellet): 1758 cm^{-1} (β-lactam carbonyl)				
12)	UV spectrum (water): λ_{max}=242 nm (ε=32300), 272 nm (ε=29530)				
13)	^1H NMR spectrum (D_2O) (δ, ppm): 3.62 (NCH_3, s, 3), 3.98 (OCH_3, s, 3). 6.99 (5-thiazolyl-H, s, 1); the corresponding shift values for the (*E*)-isomer of Ro 13-9904/001 are 3.65, 4.11 and 7.58, respectively.				

* Dedicated to Dr. OTTO ISLER on the occasion of his 70th birthday.

Table 2. *In vitro* activity of Ro 13-9904/001, some structural analogues, and reference compounds (MIC μg/ml).
Medium: DST agar (Oxoid); (*Haemophilus, Neisseria*: DST chocolate agar)
Inoculum: $\sim 10^5$ cells/spot (applied with a multipoint inoculator)

	*E. coli** R 2191	*Serratia** *marcescens* 803–15	*Enterob.** *cloacae* KA 1	*Proteus** *mirabilis* 2117	*Proteus** *vulgaris* 8313	*Pseudom.** *aeruginosa* 2100	*Pseudom.** *aeruginosa* 143 738	*Haemoph. influenzae* 3457	*Neisseria gonorrhoeae* BA 10	*Staphyl. aureus* ATCC 6538
Ro 13-9904/001	0.08	0.16	5	0.01	0.02	10	10	0.005	0.0025	5
Ro 15-0197/001	0.04	0.16	1.2	0.04	0.02	40	80	0.01	0.01	2.5
Ro 14-7746/001	0.04	0.16	2.5	0.04	0.02	40	40	0.02	0.02	2.5
Ro 14-6787/001	0.08	0.32	2.5	0.08	0.02	80	80	0.02	0.02	2.5
Ro 14-1689/001	0.04	0.16	2.5	0.08	0.02	80	80	0.01	0.02	1.2
Ro 14-8314/001	0.08	0.16	10	0.01	0.01	40	40	0.01	0.0025	10
Cefotaxime	0.04	0.16	5	0.04	0.04	20	20	0.02	0.01	2.5
SCE-1365	0.02	0.16	2.5	0.04	0.02	40	20	0.01	0.005	1.2
Cefamandole	1.2	40	40	2.5	40	>160	>160	0.63	0.16	0.16
Cefazolin	2.5	>80	>80	20	>80	>160	>160	10	2.5	0.16

* β-lactamase-producing strains

SCE-1365[3] (Fig. 1), have been shown to possess excellent antibacterial properties but relatively short plasma half-lives. We have demonstrated that suitable modification of the 3-heterocyclic-thiomethyl moiety markedly affects the plasma half-life.

In this paper we report on Ro 13-9904/001, (6R,7R)-7-{2-(2-amino-4-thiazolyl)-2-[(*Z*)-methoxyimino] acetamido}-3-{[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-yl)thio] methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid disodium salt, and some structural congeners. The synthesis of Ro 13-9904/001 is outlined in Scheme 1.

Physico-chemical properties and analytical data of Ro 13-9904/001 suitable for characterization and identification are summarized in Table 1.

The potent *in vitro* activity of Ro 13-9904/001 against a number of representative bacterial strains in comparison with the activities of several structural analogues (Fig. 2) and the reference compounds cefotaxime*, SCE-1365*, cefamandole, and cefazolin, is presented in Table 2.

Ro 13-9904/001 was found to be highly active against a wide range of both β-lactamase-producing and non-producing Gram-negative organisms. Furthermore, excellent activity was displayed against gentamicin-resistant Gram-negative strains (Table 3).

Ro 13-9904/001 showed very low acute toxicity, the LD_{50} in mice and rats being *ca.* 2,200 mg/kg following i.v. administration.

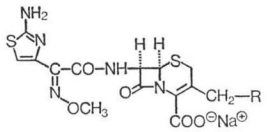
The *in vivo* activity of Ro 13-9904/001 proved to be much higher than that of cefotaxime and SCE-1365 in infection models where the antibiotic was administered 2 hours before the animals were challenged with the pathogen (Table 4). These infection models were designed to identify antibiotics with a longer plasma half-life.

In contrast, the structural analogues of Ro 13-9904/001 listed in Fig. 2 behaved like cefotaxime and SCE-1365 in models where challenge with the pathogen was delayed.

The long-acting efficacy of Ro 13-9904/001 was concluded to be the consequence of its long plasma half-life, as the plasma half-life of Ro 13-9904/001 in rats was demonstrated to be significantly longer than that of its structural analogues,

* Both cefotaxime and SCE-1365 were synthesized in our laboratories for comparison purposes.

Fig. 2. Structure of analogues of Ro 13-9904/001.



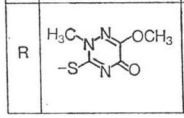
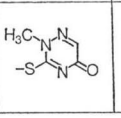
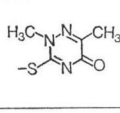
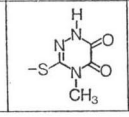
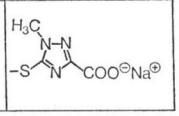
No.	Ro 15-0197/001	Ro 14-7746/001	Ro 14-6787/001	Ro 14-1689/001	Ro 14-8314/001
R					

Table 3. *In vitro* activity of Ro 13-9904/001 against gentamicin-resistant strains (MIC μg/ml). Medium: DST agar (Oxoid). Inoculum: $\sim 10^5$ cells/spot (applied with a multipoint inoculator)

	Pseudomonas aeruginosa PA 3	*Providencia stuartii* PS 1	*Proteus rettgeri* R 31	*E. coli* 5/9 B	*Serratia marcescens* 70147
Ro 13-9904/001	6.3	0.003	0.05	0.19	0.39
Gentamicin	>50	50	25	12.5	50

Table 4. Activity of Ro 13-9904/001, cefotaxime, and SCE-1365 in experimental septicemia of mice.

Dosage time* (hours)	ED_{50} (mg/kg s.c.)					
	Serratia marcescens 803-15			*Proteus mirabilis* 2117		
	Ro 13-9904/001	Cefotaxime	SCE-1365	Ro 13-9904/001	Cefotaxime	SCE-1365
+1/+3	0.08	0.15	0.13	0.02	0.05	0.12
−2	0.18	>10	>10	<0.10	>10	>10

* +: administration after bacterial challenge
−: administration prior to bacterial challenge

Table 5. Plasma half-life of Ro 13-9904/001, some structural analogues, cefotaxime, and SCE-1365 in the rat after i.v. administration of 20 mg/kg*.

Compound	$t_{1/2}$ (min.)
Ro 13-9904/001	35
Ro 15-0197/001	12
Ro 14-7746/001	10
Ro 14-6787/001	12
Ro 14-1689/001	10
Ro 14-8314/001	22
Cefotaxime	14
SCE-1365	14

* Plasma levels were determined by a microbiological assay using *B. subtilis* ATCC 6633 as the test organism.

as well as cefotaxime and SCE-1365 (Table 5).

Comparing the structures of the substituents at position 3 of these cephalosporins, it is assumed that the longer half-life of Ro 13-9904/001 is due to the presence of the enolate anion at the triazine moiety. Ro 14-8314/001, an isomer of Ro 13-9904/001 bearing a carboxylate anion at the triazole moiety, shows an intermediate half-life between that of Ro 13-9904/001 and those of the remaining cephalosporins which lack an anion-forming group at the 3-substituent.

In conclusion, Ro 13-9904/001 has been shown to be a potent, long-acting parenteral antibiotic with a very broad spectrum of activity.

Further detailed data regarding antibacterial, pharmacokinetic and toxicological aspects of the cephalosporin Ro 13-9904/001, which is currently undergoing clinical trials, will be published elsewhere.

Summary

Ro 13-9904/001 is a novel parenteral cephalosporin antibiotic with potent *in vitro* activity against a wide range of β-lactamase-producing and non-producing pathogens. In addition, Ro 13-9904/001 proved to possess a very long plasma half-life and, as a consequence, high prophylactic *in vivo* effectiveness.

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References

1) BUCOURT, R.; R. HEYMÈS, A. LUTZ, L. PÉNASSE & J. PERRONET: Propriétés antibiotiques inattendues dans le domaine des céphalosporines. C. R. Acad. Sc. Paris, Série D, 284: 1847~1849, 1977
2) HEYMÈS, R.; A. LUTZ & E. SCHRINNER: Experimental evaluation of HR 756, a new cephalosporin derivative: Pre-clinical study. Infection 5: 259~260, 1977
3) OCHIAI, M.; O. AKI, A. MORIMOTO, T. OKADA & Y. MATSUSHITA: New cephalosporin derivatives with high antibacterial activities. Chem. Pharm. Bull. 25: 3115~3117, 1977