SYNTHESIS AND BIOLOGICAL PROPERTIES OF 7β-[(Z)-2-(2-AMINO-4-THIAZOLYL)-4-CARBOXY-2-BUTENOYLAMINO]-3-CEPHEM-4-CARBOXYLIC ACID (7432-S), A NEW ORAL CEPHEM ANTIBIOTIC

Sir:

In contrast to numerous improvements in parenteral β-lactam antibiotics, progress has been little successful in development of oral β-lactam antibiotics since cephalexin was brought to the market[1]. Recently, a new type of orally absorbable cephem FK027 (cefixime)[2] has been reported which possesses the (*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido group as the 7β-acyl side chain that structurally differs from the arylglycylamino group in cephalexin and its analogs. During the course of our research on orally active cephem derivatives, we have selected 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-4-carboxy-2-butenoylamino]-3-cephem-4-carboxylic acid (7432-S) as the clinical candidate having unique antibacterial activity and excellent oral bioavailability (more than 80% in healthy volunteers[3]. In this communication we describe synthesis of 7432-S (**9a**) and its analogs (**9b** and **9c**) together with some biological properties.

Compounds **9a**, **9b** and **9c** were synthesized as outlined in Scheme 1. Diphenylmethyl 2-(2-benzyloxycarbonylamino-4-thiazolyl)acetate (**1**) was reacted with diphenylmethyl formate (**2**) in the presence of sodium hydride (THF, 0~20°C, 2 hours) to give 2-hydroxymethylene acetate **3** in good yield. Wittig reaction of **3** with phosphorane **4** gave diester **5** as an isomeric mixture ($E/Z \fallingdotseq 3/2$), which was treated with TFA in CH_2Cl_2 (0~5°C, 1.5 hours) affording mono acids **6** as a mixture. Coupling reaction of **6** with 7β-aminocephem **7a** by the usual method ($POCl_3$, *N*-methylmorpholine, −20°C, 20 minutes in CH_2Cl_2) gave cephem esters **8a** as an isomeric mixture ($E/Z \fallingdotseq 85/15$). Deprotection of **8a** with aluminum trichloride-anisole gave a mixture of diacids **9a** and **10** in the almost same isomeric *E*/*Z* ratio as that for the esters **8a**. The desired (*Z*)-isomer **9a** ($E/Z \fallingdotseq 1/99$) was isolated from the mixture in good yield by slow crystallization from an aq solution (pH 3~4) with concomitant isomerization. (*E*)-Isomer **10** could be obtained as the ammonium salt only by a tedious isolation process using preparative HPLC (Lichroprep. RP 18) eluted with 0.01 M

Scheme 1.

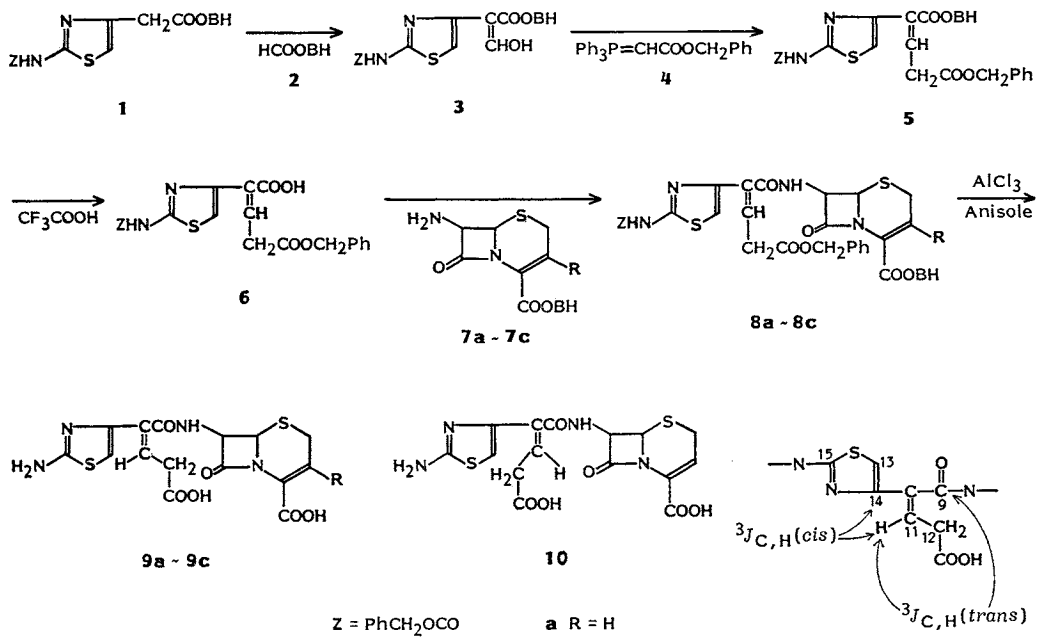

Table 1. *In vitro* antibacterial activity (MIC, μg/ml) of compounds **9a**, **9b**, **9c** and cefaclor.

Organism	9a	9b	9c	Cefaclor
Staphylococcus aureus 209-P JC-1	100	12.5	25	0.39
S. epidermidis ATCC 14990	12.5	6.25	12.5	0.78
Streptococcus pyogenes C 203	0.39	0.1	0.2	0.1
Haemophilus influenzae SR3508*	0.1	0.39	0.1	3.13
Escherichia coli NIHJ JC-2	0.1	0.78	0.1	3.13
E. coli SR73*	0.39	3.13	0.2	12.5
Klebsiella pneumoniae SR1	0.013	0.1	0.013	0.78
Proteus mirabilis PR4	0.025	0.05	0.025	1.56
P. vulgaris CN329	0.025	0.05	0.025	50
Enterobacter cloacae SR233	0.39	1.56	0.1	100
Serratia marcescens ATCC 13880	0.1	0.78	0.1	>100
Pseudomonas aeruginosa SR24	100	>100	25	>100

* ABPC-resistant strain.

Table 2. Bioavailability of compounds **9a**, **9b**, **9c** and cefaclor after oral dosing of 20 mg/kg.

Animal[a]	Parameter[b]	9a (7432-S)	9b	9c	Cefaclor
Mouse	Cmax	16.7	22.1	7.2	14.9
	AUC	20.4	36.6	13.2	10.5
	UR	55.7	46.9	27.1	48.8
Monkey	Cmax	7.2	7.8	3.3	9.2
	AUC	33.9	41.9	11.3	17.7
	UR	20.2	20.5	6.4	78.8

[a] Mouse: Jcl-ICR, male, 6 weeks ($n=5$), monkey: Cynomolgus, female ($n=3\sim8$).

[b] Parameters represent mean value, Cmax (μg/ml): Maximum of plasma level, AUC (μg·hours/ml): The area under plasma level-time curve, UR (%): Recovery in urine for 2 hours in mice and for 24 hours in monkeys.

ammonium acetate. Compounds **9b** and **9c** also were prepared by a process similar to that used for **9a**. Structural determination of **9a**†, and **10**†† is based on ^{1}H NMR chemical shifts of vinylic 11-H (relative chemical shift value: $Z>E$) and ^{13}C-^{1}H three-bond coupling constants between C-14 and vinylic 11-H (6 Hz, in **9a**; 10 Hz in **10**) as well as between amide C-9 and 11-H (12 Hz in **9a**; 7 Hz in **10**). Analogously, structural assignment for **9b** and **9c** were made from ^{1}H NMR chemical shifts of vinylic 11-H.

Table 1 shows antibacterial activity of **9a**, **9b** and **9c** as compared with that of cefaclor. Every derivative displayed potent activity with widely expanded spectra against Gram-negative bacteria. Against Gram-positive bacteria, streptococcal activity was comparable to that of cefaclor although the staphylococcal activity was only marginal. The 3-carbamoyloxymethyl derivative **9c** is the most active, followed by **9a**. Compound **9b** was weak against Gram-negative

† Analytical data and ^{1}H NMR of **9a** disodium are as follows: *Anal* Calcd for $C_{15}H_{12}N_4O_6S_2Na_2\cdot2\frac{1}{2}H_2O$: C 36.07, H 3.43, N 11.22, S 12.84, Na 9.21, H_2O 9.02. Found: C 36.22, H 3.45, N 11.35, S 12.80, Na 9.10, H_2O 8.62. ^{1}H NMR (200 MHz, D_2O) δ 3.22 (2H, d, J=8.0 Hz, CHCH_2CO), 3.51 (1H, dd, J=18.9 and 6.0 Hz, SC$H_2(\beta)$), 3.69 (1H, dd, J=18.9 and 2.7 Hz, SC$H_2(\alpha)$), 5.20 (1H, d, J=4.7 Hz, 6-H), 5.84 (1H, d, J=4.7 Hz, 7-H), 6.32 (1H, dd, J=6.0 and 2.7 Hz, 3-H), 6.50 (1H, t, J=8.0 Hz, CHCH$_2$CO), 6.60 (1H, s, thiazole 5-H): $^3J_{C,H}$ (C-9, 11-H=12.0 Hz, *trans*; C-14, 11-H=6.0 Hz, *cis*).

†† ^{1}H NMR data of **10** ammonium are as follows: (200 MHz, D_2O) δ 3.26 (2H, d, J=8.0 Hz, CHCH_3CO), 3.48 (1H, dd, J=18.9 and 6.0 Hz, SC$H_2(\beta)$), 3.66 (1H, dd, J=18.9 and 2.7 Hz, SC$H_2(\alpha)$), 5.15 (1H, d, J=4.7 Hz, 6-H), 5.77 (1H, d, J=4.7 Hz, 7-H), 6.31 (1H, dd, J=6.0 and 2.7 Hz, 3-H), 6.77 (1H, s, thiazole 5-H), 6.94 (1H, t, J=8.0 Hz, CHCH$_2$CO): $^3J_{C,H}$ (C-9, 11-H=7.0 Hz, *cis*; C-14, 11-H=10.0 Hz, *trans*).

bacteria. *Pseudomonas aeruginosa* was less susceptible than Enterobacteriaceae to three compounds.

Oral bioavailability of these compounds are listed in Table 2. The 3-vinyl derivative **9b** showing the weaker Gram-negative activity was found to have the best absorbability, followed by **9a**. Compound **9c** was the highest in antibacterial activity, but the oral absorption was too poor to be qualified for further evaluation. A favorable profile was found in compound **9a** by considering potent Gram-negative activity and good bioavailability.

Thus, 3-unsubstituted derivative **9a**, designated as 7432-S, was selected for clinical evaluation.

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