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Studies on Novel 3-Isoxazolylvinylcephalosporins:

II. Synthesis and Biological Activity of 7-[2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido] Derivatives

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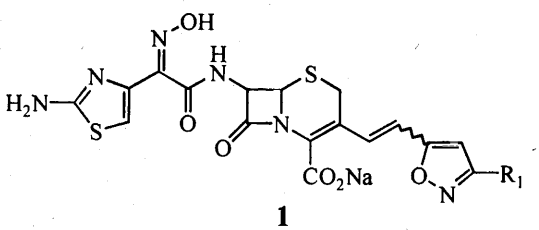
In the previous paper, we found that 7-[2-(2-aminothiazol-4-yl)-2-(alkoxy)iminoacetamido]-3-[3-(substituted)isoxazolyl]vinylcephalosporins showed increased antibacterial activity against Gram-positive bacteria. Variation of the alkyl group of the oxime functionality at the C-7 side chain of the cephalosporins from methyl to cyclopentyl, though the pharmacokinetic profile got worse, improved their activity especially against Gram-positive bacteria. Based on those results, we prepared the cephalosporin compounds of the general structure **1** by replacing the alkyl group of the oxime with hydrogen, and evaluated their biological activity focusing on Gram-positive bacteria. And also the C-4 ester prodrugs were prepared and their oral availability was investigated.

The synthesis of the cephalosporin compounds **1a**~**k** is outlined in Scheme 1. It utilizes basically the same protocol adopted in the preparation of 7-[2-(2-aminothiazol-4-yl)-2-(alkoxy)iminoacetamido]-3-[3-(substituted)isoxazolyl]vinylcephalosporins in the previous paper. Acylation of 7-amino-3-chloromethylcephalosporanic acid (**3**) with masked 2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetic acid (**2**) afforded the key intermediate **4**, which then could be coupled with the isoxazole aldehyde **6**[1,2] *via* the phosphonium salt **5** to produce the fully protected product **7** as a mixture of geometrical isomers ($Z:E\sim9:1$). Through the sequential deprotection by trifluoroacetic acid and formic acid gave the crude free acid form of the final product **1**. Sodium salt formation by the treatment with aq sodium bicarbonate solution followed by the purification by reverse phase column chromatography (LiChrosorb® RP-18, 25% aq. MeOH) and freeze drying yielded the pure product **1** ready for biological evaluation. [(*Z*)-**1g**: ^{1}H NMR (300 MHz, D_2O) δ 3.40 (1H, d, J=18.0 Hz, C2-H), 3.68 (1H, d, J=18.0 Hz, C2-H), 3.95 (3H, s, $-OCH_3$), 5.38 (1H, d, J=4.8 Hz, C6-H), 5.90 (1H, d, J=4.8 Hz, C7-H), 6.09 (1H, s, isoxazole-H), 6.40 (1H, d, J=12.2 Hz, vinyl-H), 6.69 (1H, d, J=12.2 Hz, vinyl-H), 7.02 (1H, s, thiazole-H); (*Z*)-**1i**: ^{1}H NMR (300 MHz, D_2O) δ 3.41 (1H, d, J=18.0 Hz, C2-H), 3.71 (1H, d, J=18.0 Hz, C2-H), 5.40 (1H, d, J=4.7 Hz, C6-H), 5.92 (1H, d, J=4.7 Hz, C7-H), 6.51 (1H, d, J=12.1 Hz, vinyl-H), 6.52 (1H, s, isoxazole-H), 6.73 (1H, d, J=12.1 Hz, vinyl-H), 7.04 (1H, s, thiazole-H)].

The *in vitro* antibacterial activities (MIC, μg/ml) of the cephalosporins prepared as mixtures of (*Z*)- and (*E*)-isomers (~9 : 1) against selected strains are summarized in Table 1. MIC values were determined by the two-fold Mueller-Hinton agar dilution method,[3] and MICs of cefpirome and cefpodoxime are also presented for comparison. Their antibacterial spectrum was similar to those of 7-[2-(2-aminothiazol-4-yl)-2-(alkoxy)iminoacetamido]-3-[3-(substituted)isoxazolyl]vinylcephalosporins reported in the previous paper. In addition, we found that their activities against Gram-positive bacteria were considerably improved. Except for the compounds **1f** and **1k**, all the compounds prepared showed comparable or superior activity to those of reference compounds against Gram-positive bacteria. Among them, the compounds **1g** and **1i** exhibited the best antibacterial activity. Both compounds showed MICs four times greater than cefpirome against *Enterococcus faecium* MD 8b. Against *Staphylococcus aureus* 285 they showed

Fig. 3-Isoxazolylvinylcephalosporins.



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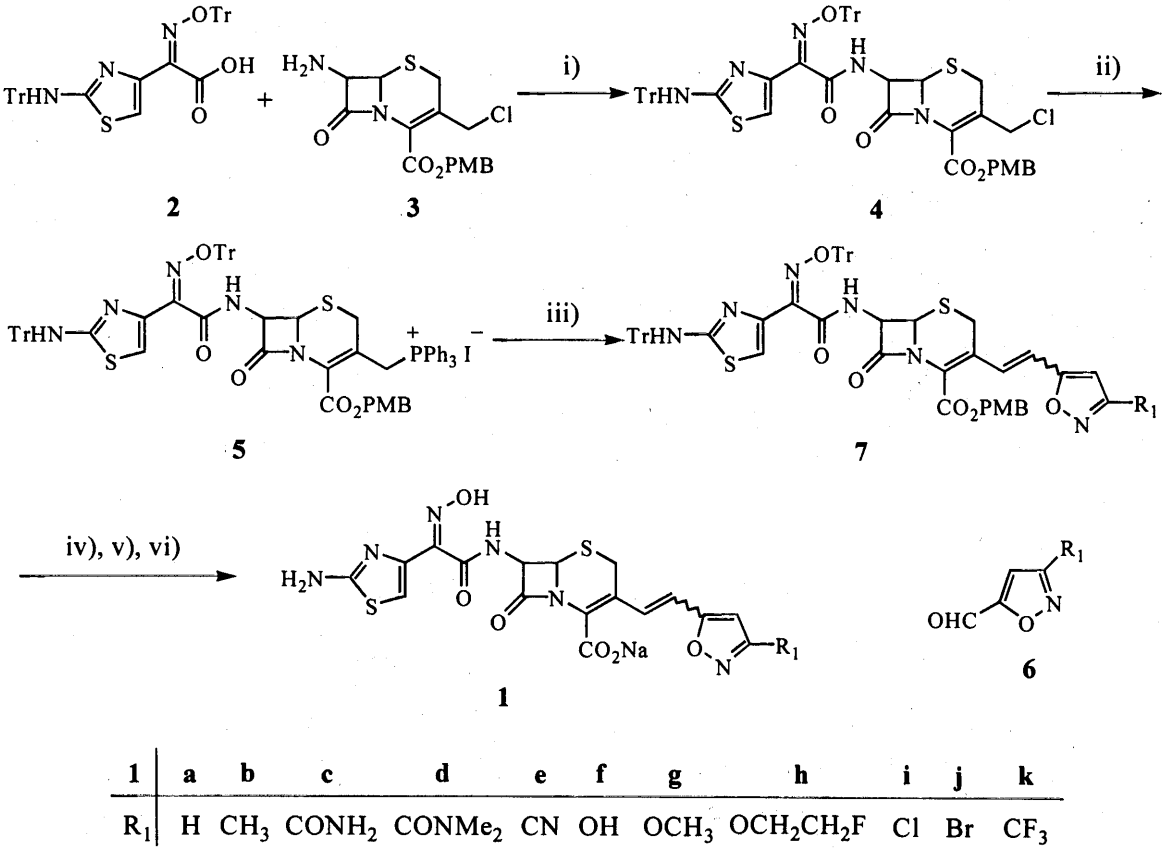
Scheme 1. Synthesis of 3-isoxazolylvinylcephalosporins.



1	a	b	c	d	e	f	g	h	i	j	k
R_1	H	CH_3	$CONH_2$	$CONMe_2$	CN	OH	OCH_3	OCH_2CH_2F	Cl	Br	CF_3

Tr = Triphenylmethyl. PMB = *p*-Methoxybenzyl.

Reaction Conditions: i) $POCl_3$, Py/CH_2Cl_2, $-5°C$ (80~86%); ii) PPh_3, NaI/acetone, rt (95~98%); iii) **6**, 2 N $NaHCO_3$/CH_2Cl_2, 0°C~rt (40~53%); iv) TFA/anisole; v) HCOOH; vi) $NaHCO_3$; RP-18 colum chromatography; freeze dry (40~42% from **7**)

Table 1. MICs of compounds **1a**~**1k** against selected strains (μg/ml)

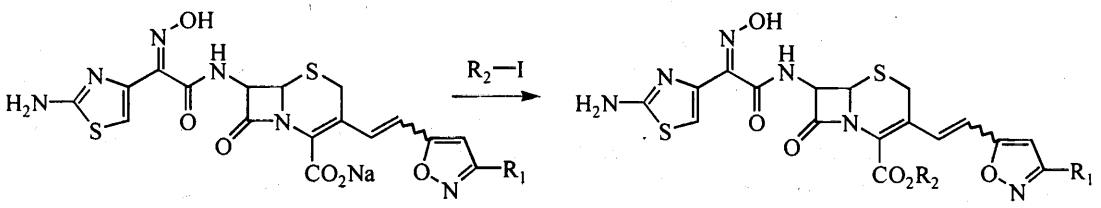
Compound	Microorganism[a]									
	S. a. 1	*S. a.* 2	*S. a.* 3	*S. p.*	*E. f.*	*E. c.*	*S. t.*	*K. o.*	*En. c.*	*P. a.*
1a	0.195	0.391	0.098	0.004	3.125	0.098	0.025	12.5	0.013	>100
1b	0.195	0.391	0.195	0.004	3.125	0.195	0.195	25	0.049	>100
1c	0.391	0.195	0.195	0.007	3.125	0.049	0.049	>100	0.013	50
1d	0.781	0.781	0.195	0.004	6.25	0.781	0.781	>100	0.049	>100
1e	0.195	0.391	0.098	0.007	6.25	0.391	0.098	50	0.049	50
1f	0.781	1.563	0.391	0.025	50	0.391	0.098	50	0.025	6.25
1g	0.195	0.195	0.098	<0.002	1.563	0.391	0.098	100	0.049	>100
1h	0.391	0.391	0.098	<0.002	3.125	0.049	0.098	50	0.025	>100
1i	0.098	0.098	0.098	<0.002	1.563	0.195	0.098	100	0.049	100
(*Z*)-**1i**	0.195	0.195	0.049	0.007	0.781	0.195	0.195	50	0.049	50
(*E*)-**1i**	0.195	0.098	0.049	0.007	0.781	0.098	0.049	50	0.013	12.5
1j	0.098	0.195	0.098	0.007	3.125	0.391	0.098	25	0.049	100
1k	0.781	0.781	0.195	0.013	50	6.25	1.563	>100	0.049	>100
Cefpirome	0.391	1.563	0.098	0.007	6.25	0.013	0.013	0.781	0.007	0.781
Cefpodoxime	1.563	3.125	1.563	0.007	50	0.195	0.098	3.125	0.098	100

[a] Microorganisms: *S. a.* 1 = *Staphylococcus aureus* SG 511; *S. a.* 2 = *Staphylococcus aureus* 285; *S. a.* 3 = *Staphylococcus aureus* 503; *S. p.* = *Streptococcus pyogenes* A 308; *E. f.* = *Enterococcus faecium* MD 8b; *E. c.* = *Escherichia coli* 1507E; *S. t.* = *Salmonella typhimurium*; *K. o.* = *Klebsiella oxytoca* 1082E; *En. c.* = *Enterobacter cloacae* 1321E; *P. a.* = *Pseudomonas aeruginosa* 1771.

Table 2. MICs of **1g** and **1i** against MRSA (μg/ml).

Strains	Compound				
	1g	**1i**	Cefdinir	Cefpodoxime	Vancomycin
Staphylococcus aureus 88 E	1.563	1.563	3.125	100	0.781
Staphylococcus aureus 208 E	0.391	0.195	0.391	3.125	0.781
Staphylococcus aureus 692 E	1.563	0.781	3.125	>100	0.781
Staphylococcus aureus 694 E	0.781	1.563	1.563	6.25	0.781
Staphylococcus aureus 695 E	0.781	1.563	0.781	3.125	0.781
Staphylococcus aureus 701 E	1.563	1.563	6.25	100	0.781
Staphylococcus aureus 705 E	0.781	0.195	1.563	3.125	0.391
Staphylococcus aureus 708 E	1.563	1.563	12.5	>100	0.781
Staphylococcus aureus 711 E	0.781	0.391	3.125	6.25	0.781
Staphylococcus aureus 714 E	0.391	0.391	0.781	6.25	0.781

Table 3. Cephalosporin esters prepared.



1 **8**

1	**8**	R_1	R_2
1g	**8g$_1$**	OCH_3	$-CH_2OCOC(CH_3)_3$
1g	**8g$_2$**	OCH_3	$-CH(CH_3)OCOOCH(CH_3)_2$
1i	**8i$_1$**	Cl	$-CH_2OCOC(CH_3)_3$
1i	**8i$_2$**	Cl	$-CH(CH_3)OCOOCH(CH_3)_2$

Table 4. Pharmacokinetic parameters of **1g**, **1i** and their ester derivatives.

Compound	Route	C_{max} (μg/ml)	T_{max} (hrs)	$T_{1/2}$ (hrs)	AUC (μg·hr/ml)	BA[a] (%)
1g	s.c.	90.76±4.88	0.36±0.06	1.37±0.12	124.02±10.29	—
8g$_1$	p.o.	27.24±5.98	0.64±0.12	1.95±0.22	64.30±11.00	51.5
8g$_2$	p.o.	16.88±1.82	0.62±0.13	2.38±0.34	46.99±6.04	37.4
1i	s.c.	108.37±7.32	0.25±0.05	1.08±0.09	156.10±29.45	—
8i$_1$	p.o.	20.99±5.39	1.33±0.41	1.64±0.26	50.99±10.57	32.5
8i$_2$	p.o.	27.68±7.77	0.67±0.11	2.38±0.36	62.34±15.20	39.9
Cefpodoxime	s.c.	39.95±4.18	0.42±0.05	0.41±0.05	43.04±3.94	—
Cefpodoxime proxetile	p.o.	30.21±3.26	1.00±0.04	0.45±0.06	36.18±3.62	84.1

[a] BA (Bioavailability) = AUC(p.o.)/AUC(s.c.).
Conditions: solvent = saline; medium = Mueller-Hinton agar; microorganism = *Streptococcus pyogenes* 77A; amount = 40 (mg/kg); animal = male ICR mice, mean body weight = ~25 g, 4 mice per group.

four times and eight times greater activity than cefpirome, respectively.

The geometry of the C-3 vinyl group seemed not to alter the antibacterial activity significantly against Gram-positive bacteria. Each of the geometrical isomers of the compound **1i**, *i.e.* (*Z*)-**1i** and (*E*)-**1i**, exhibited activity similar to that of the mixture (*Z* : *E* ~ 9 : 1) against Gram-positive bacteria, while the (*E*)-isomer showed considerable enhancement in activity against *Pseudomonas aeruginosa*.

To further evaluate their potency, MICs of the compounds **1g** and **1i** against MRSA were determined. Against MRSA strains, they showed slightly less activity than vancomycin, but were more potent than the other reference compounds, cefdinir and cefpodoxime. The results are summarized in Table 2.

The pivaloyloxymethyl and 1-isopropoxycarbonyloxyethyl esters of the compounds **1g** and **1i** were prepared (Table 3) and their pharmacokinetic profiles were characterized (Table 4). AUC values of the parent compounds **1g** and **1i** were exceptionally large when compared to that of cefpodoxime, owing to their high maximum concentrations and long duration times. Those unusual pharmacokinetic parameters seemed partly due to their high level of serum protein binding. Serum protein binding analysis by the ultrafiltration method revealed that the compounds **1g** and **1i** bound to dog serum protein by 99% and 88%, respectively. Bioavailability of the ester derivatives was 51.5% (**8g**$_1$) at best, but the AUC values were still large enough to be administered orally.

Acknowledgments

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References

1) TAYLOR, E. C. & P. S. RAY: Pteridines. 54. A novel synthetic approach to C-6 carbon substituted pterins via intermolecular 1,3-dipolar cycloaddition reactions. J. Org. Chem. 56: 1812 ~ 1816, 1991
2) CHIARINO, D.; M. NAPOLETANO & A. SALA: One pot synthesis of 3-chloro-5-substituted isoxazoles by 1,3-dipolar cycloaddition. Synth. Commun. 18: 1171 ~ 1176, 1988
3) LEITNER, F.; M. MISIEK, T. A. PURSIANO, R. E. BUCK, D. R. CHISHOLM, R. G. DEREGIS, Y. H. TSAI & K. E. PRICE: Laboratory evaluation of BL-S 786, a cephalosporin with broad-spectrum antibacterial activity. Antimicrob. Agents Chemother. 10: 426 ~ 435, 1976