Studies on Novel 3-Isoxazolylvinyl-cephalosporins:

I. Synthesis and Biological Activity of 7-[2-(2-Aminothiazol-4-yl)-2-(alkoxy)-iminoacetamido] Derivatives

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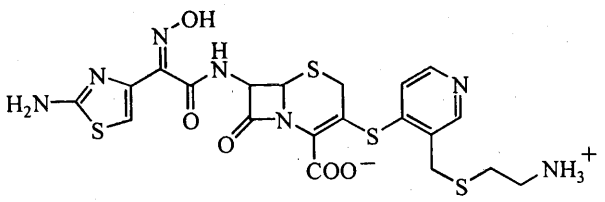
The prevalence of various resistant strains including MRSA (methicillin-resistant *Staphylococcus aureus*) has led researchers to focus on the antibiotics effective against specific pathogens or specific groups of bacteria, rather than the broad spectrum ones, to minimize the probability of evoking new types of resistant strains. The cephalosporins of Microcide (MC-02,479),[1,2] Bristol-Myers Squibb[3] and Meiji Seika[4] are examples that focused on Gram-positive bacteria including MRSA (Fig. 1).

Recently we reported the synthesis and biological activity of a series of cephalosporin compounds having a catechol moiety attached *via* a vinyl linkage at the C-3 position.[5] There we found that the introduction of an isoxazole skeleton between the vinyl and the catechol group enhanced the antibacterial activity of the cephalosporin compounds, especially against Gram-positive bacteria. Encouraged by this result, we decided to decrease their antibacterial spectrum to Gram-positive bacteria by concentrating on the isoxazole group attached directly to the C-3 vinyl group.

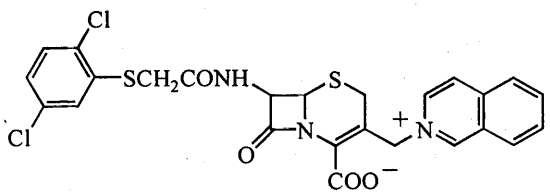
Novel 3-isoxazolylvinylcephalosporin compounds of general structure **1** were prepared and tested for biological activities (Fig. 2). Also the ester derivatives of selected compounds were prepared to examine their oral absorbability.

Protected 2-(2-aminothiazol-4-yl)-2-(alkoxy)iminoacetic acid **2** was reacted with *p*-methoxybenzyl 7-amino-3-chloromethylcephalosporanate (**3**) by a conventional method ($POCl_3$, Py/CH_2Cl_2, $-5°C$) to give an acylated product **4** in 80~85% yield (Scheme 1). [**4**, $R_1=CH_3$: ^1H NMR (300 MHz, $CDCl_3$) δ 3.38 (1H, d, $J=18.1$ Hz, C2-H), 3.64 (1H, d, $J=18.1$ Hz, C2-H), 3.81 (3H, s, $-OCH_3$), 4.07 (3H, s, $-NOCH_3$), 4.43 (1H, d, $J=11.8$ Hz, C3′-H), 4.59 (1H, d, $J=11.8$ Hz, C3′-H), 5.03 (1H, d, $J=4.8$ Hz, C6-H), 5.22 (2H, s, $-OCH_2-$), 5.92 (1H, dd, $J_1=4.8$ Hz, $J_2=8.7$ Hz , C7-H), 6.74 (1H, s, thiazole-H), 6.77 (1H, d, $J=8.7$ Hz, NH), 6.90 (2H, d, $J=8.4$ Hz, Ph), 7.20~7.39 (17H, m, Ph)]. It was then converted to a phosphonium salt **5** (PPh_3, NaI/acetone, rt, 95~98%), followed by the reaction (2 N $NaHCO_3/CH_2Cl_2$, 0°C~rt) with a substituted isoxazole aldehyde **6**, usually prepared by the sequential reduction-oxidation of the 1,3-dipolar cycloaddition[6,7] product of methyl propiolate and the

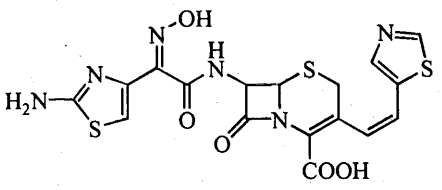
Fig. 1. Cephalosporins focused on Gram-positive bacteria.



Microcide (MC-02,479)

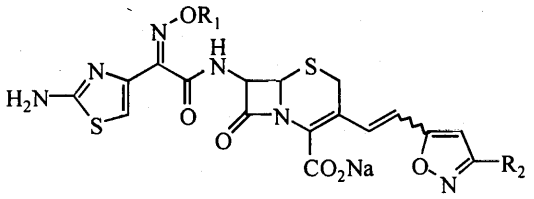


Bristol-Myers Squibb



Meiji Seika

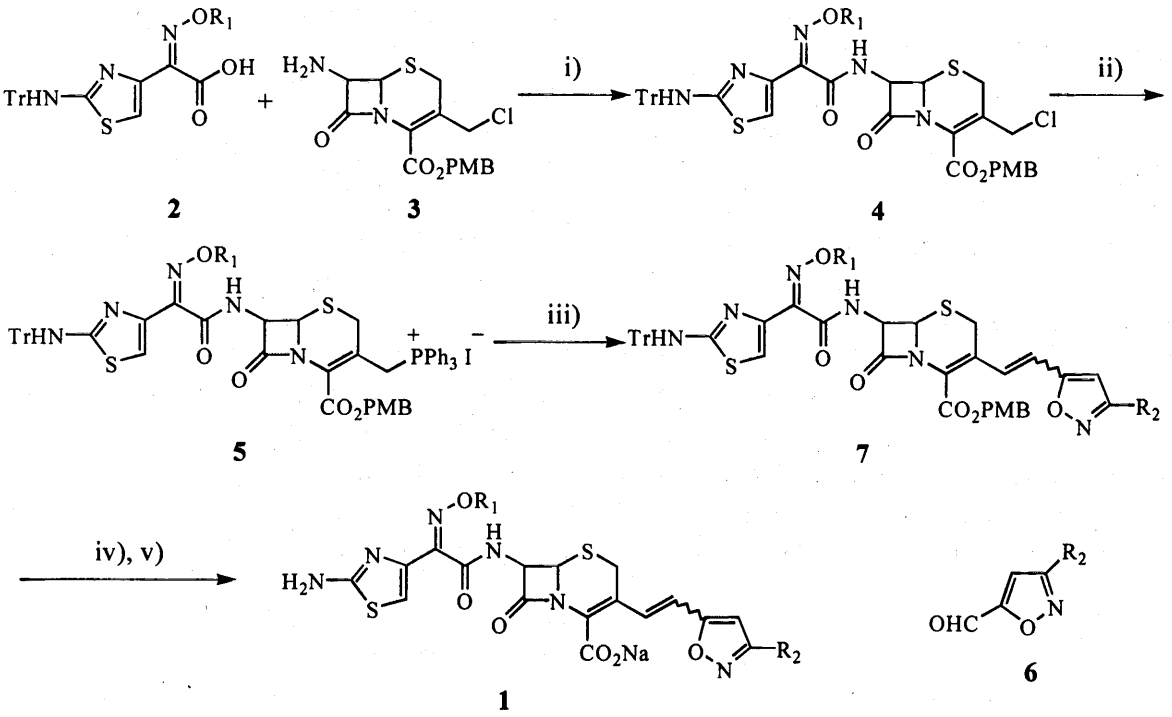
Fig. 2. General structure of 3-isoxazolylvinylcephalosporins.



1a~t

$R_1 = CH_3$, cyclopentyl
R_2 = H, CH_3, CH_2CONH_2, CH_2CONMe_2, CH_2CONH_2, CH_2CONMe_2, CO_2Na, CN, OH, OCH_3, OCH_2CH_2F, Cl, Br, CF_3

Scheme 1. Synthesis of novel 3-isoxazolylvinylcephalosporins.



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

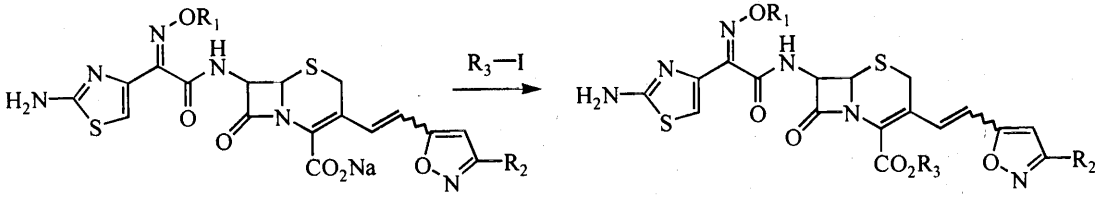
Reaction conditions: i) $POCl_3$, Py/CH_2Cl_2, −5°C (80~86%); ii) PPh_3, NaI/acetone, rt (95~98%); iii) **6**, 2N $NaHCO_3$/CH_2Cl_2, 0°C~rt (40~53%); iv) TFA/anisole; v) $NaHCO_3$: RP-18 column chromatography; freeze dry (40~42% from **7**).

corresponding aldoxime, to afford the protected final product **7**, predominantly in (*Z*)-form, in 40~53% yield (*Z*:*E*~9:1). [**6**, R_2 = Cl: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (1H, s, isoxazole-H), 9.94 (1H, s, −CHO); (*Z*)-**7**, $R_1 = CH_3$, R_2 = Cl: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.32 (1H, d, J = 18.0 Hz, C2-H), 3.59 (1H, d, J = 18.0 Hz, C2-H), 3.81 (3H, s, $-OCH_3$), 4.08 (3H, s, $-NOCH_3$), 5.10~5.16 (3H, m, C6-H, $-OCH_2-$), 5.98 (1H, dd, J_1 = 4.5 Hz, J_2 = 9.0 Hz, C7-H), 6.13 (1H, s, isoxazole-H), 6.38 (1H, d, J = 12.0 Hz, vinyl-H), 6.75 (1H, d, J = 12.0 Hz, vinyl-H), 6.88 (2H, d, J = 8.0 Hz, Ph), 7.02 (1H, s, thiazole-H), 7.20~7.42 (18H, m, Ph, NH)]. Both geometrical isomers could be separated by flash column chromatography (*n*-hexane : EtOAc = 2 : 1) at this stage, when needed. Deprotection by TFA/anisole system, treatment with $NaHCO_3$ and purification by reverse phase column chromatography (LiChrosorb® RP-18, 25% aq. MeOH) followed by freeze drying gave the final

Table 1. 3-Isoxazolylvinylcephalosporins **1**.

Compound	R_1	R_2	Compound	R_1	R_2
1a	CH_3	H	**1k**	CH_3	OCH_2CH_2F
1b	CH_3	CH_3	**1l**	CH_3	Cl
1c	CH_3	CH_2CONH_2	**1m**	CH_3	Br
1d	CH_3	CH_2CONMe_2	**1n**	CH_3	CF_3
1e	CH_3	$CONH_2$	**1o**	Cyclopentyl	H
1f	CH_3	$CONMe_2$	**1p**	Cyclopentyl	CH_3
1g	CH_3	COONa	**1q**	Cyclopentyl	$CONH_2$
1h	CH_3	CN	**1r**	Cyclopentyl	$CONMe_2$
1i	CH_3	OH	**1s**	Cyclopentyl	Cl
1j	CH_3	OCH_3	**1t**	Cyclopentyl	Br

Table 2. Cephalosporin esters.



1 **8**

1	**8**	R_1	R_2	R_3
1a	$\mathbf{8a_1}$	CH_3	H	$-CH_2OCOC(CH_3)_3$
1a	$\mathbf{8a_2}$	CH_3	H	$-CH(CH_3)OCOCH_3$
1a	$\mathbf{8a_3}$	CH_3	H	$-CH(CH_3)OCOOCH(CH_3)_2$
1l	$\mathbf{8l_2}$	CH_3	Cl	$-CH(CH_3)OCOCH_3$
1l	$\mathbf{8l_3}$	CH_3	Cl	$-CH(CH_3)OCOOCH(CH_3)_2$
1m	$\mathbf{8m_3}$	CH_3	Br	$-CH(CH_3)OCOOCH(CH_3)_2$
1o	$\mathbf{8o_1}$	Cyclopentyl	H	$-CH_2OCOC(CH_3)_3$
1s	$\mathbf{8s_1}$	Cyclopentyl	Cl	$-CH_2OCOC(CH_3)_3$

product **1** as an amorphous white solid in 40~42% yield. [(*Z*)-**1l**: ^{1}H NMR (300 MHz, D_2O) δ 3.42 (1H, d, J=17.9 Hz, C2-H), 3.72 (1H, d, J=17.9 Hz, C2-H), 4.03 (3H, s, $-NOCH_3$), 5.40 (1H, d, J=4.6 Hz, C6-H), 5.90 (1H, d, J=4.6 Hz, C7-H), 6.51 (1H, d, J=12.0 Hz, vinyl-H), 6.52 (1H, s, isoxazole-H), 6.77 (1H, d, J=12.0 Hz, vinyl-H), 7.07 (1H, s, thiazole-H)]. These cephalosporins are shown in Table 1. The ester-type prodrugs of selected compounds could be obtained by reacting the corresponding alkyl halides with the sodium salt of the cephalosporins. They are shown in Table 2.

In vitro antibacterial activities (MIC, μg/ml) of the two series of 3-isoxazolylvinylcephalosporins as mixtures of (*Z*)- and (*E*)-isomers (~9:1) were determined by the two-fold Mueller-Hinton agar dilution method,[8] and the results for selected strains are summarized in Table 3. MICs of cefpirome and cefpodoxime are also presented for comparison.

In Table 3, most of the compounds prepared showed moderate to good activity against the Gram-positive bacteria tested, and showed relatively lower activity against the Gram-negative ones. When compared with reference compounds, cefpirome and cefpodoxime, they exhibited intermediate antibacterial activities between the two. In general, halogen substituents on the isoxazole ring conferred greater activity to the parent cephalosporins. The electronic effect of the substituent R_2 on activity is not clear. Comparing the pairs of the two series of compounds having common R_2, *i.e.* **1a~1o**, **1b~1p**, **1e~1q**, **1f~1r**, **1l~1s** and **1m~1t**, the series having R_1=cyclopentyl showed similar or two to four times better activity than the series having R_1=methyl against Gram-positive bacteria, while the tendency was reversed against Gram-negative bacteria. Focusing on Gram-

Table 3. MICs of compounds **1a**~**1t** 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	0.781	1.563	0.391	0.007	12.5	0.195	0.049	6.25	0.049	12.5
1b	0.781	1.563	0.781	0.004	25	0.391	0.098	12.5	0.049	25
1c	3.125	1.563	N. D.	0.004	50	0.195	0.049	25	0.025	25
1d	3.125	1.563	1.563	0.007	25	0.781	0.391	>100	0.195	12.5
1e	0.781	0.781	0.781	0.004	50	0.195	0.098	50	0.049	12.5
1f	3.125	3.125	0.781	0.007	50	0.195	0.098	50	0.049	50
1g	25	12.5	12.5	0.049	>100	0.195	0.098	50	0.049	25
1h	0.781	1.563	0.391	0.013	50	0.391	0.098	50	0.049	25
1i	25	12.5	6.25	0.098	>100	0.391	0.391	12.5	0.098	>100
1j	1.563	1.563	0.391	0.007	25	0.195	0.195	12.5	0.098	50
1k	0.391	0.781	0.195	0.004	25	0.391	0.391	12.5	0.098	12.5
1l	0.781	0.781	0.195	0.004	6.25	0.195	0.098	25	0.049	25
1m	0.781	0.391	0.195	0.004	12.5	0.195	0.049	25	0.195	12.5
1n	1.563	3.125	0.781	0.013	100	0.781	1.563	>100	0.013	>100
1o	0.391	0.391	0.195	<0.002	3.125	0.781	0.781	6.25	0.391	6.25
1p	1.563	0.781	0.391	0.004	6.25	1.563	0.781	6.25	1.563	25
1q	0.781	0.781	0.391	0.007	12.5	1.563	1.563	25	0.391	12.5
1r	1.563	3.125	0.781	0.007	25	1.563	0.781	50	0.781	25
1s	0.195	0.391	0.195	<0.002	3.125	0.781	0.391	6.25	0.391	12.5
1t	0.195	0.391	0.098	0.004	12.5	0.781	0.391	25	0.391	6.25
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 4. Pharmacokinetic parameters of cephalosporins selected.

Compound	Route	C_{max} (μg/ml)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC (μg·hr/ml)	BA[a] (%)
1a	s.c.	15.44±0.73	0.29±0.04	0.35±0.03	11.84±1.36	—
8a$_1$	p.o.	2.06±0.22	<0.17	0.72±0.12	2.23±0.43	18.8
8a$_2$	p.o.	1.44±0.08	<0.17	0.56±0.03	1.28±0.08	10.8
8a$_3$	p.o.	2.35±9.28	0.42±0.05	0.62±0.09	2.79±0.47	23.6
1l	s.c.	62.52±6.29	<0.17	0.94±0.07	70.09±5.04	—
8l$_2$	p.o.	8.85±0.23	0.21±0.04	1.49±0.10	15.37±0.98	21.9
8l$_3$	p.o.	12.43±2.10	0.64±0.20	1.65±0.10	29.86±5.40	42.6
1m	s.c.	66.15±6.07	<0.17	0.94±0.03	64.39±8.54	—
8m$_3$	p.o.	12.12±1.64	<0.17	1.56±0.22	17.79±0.33	27.6
1o	s.c.	5.28±0.62	<0.17	0.27±0.04	2.17±0.06	—
8o$_1$	p.o.	N.D.[b]	N.D.	N.D.	N.D.	—
1s	s.c.	8.91±0.72	<0.17	0.29±0.08	2.53±0.08	—
8s$_1$	p.o.	N.D.	N.D.	N.D.	N.D.	—
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.). [b] N.D. = Not Determined.

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.

positive bacteria, except for **1g** and **1i**, all the compounds prepared showed relatively good activity against *Staphylococcus aureus* strains. Against *Enterococcus faecium* MD 8b, compounds having R_2 = H or Cl showed superior activity to the rest of the compounds prepared. In a separate test, the compound **1m** showed intermediate activity between that of vancomycin and cefpirome against MRSA [28 strains, MIC (μg/ml) range; vancomycin: 0.391~0.781, **1m**: 0.781~12.5, cefpirome: 1.563~50].

The pharmacokinetic parameters of selected compounds are shown in Table 4. According to the results, the compounds **1l** and **1m** reached maximum concentration in a short time and maintained the level for a longer period than cefpodoxime. However, the compounds **1o** and **1s**, which showed the best antibacterial activity against Gram-positive bacteria among the compounds prepared, exhibited poor pharmacokinetic profiles. When administered orally as esters, derivatives of **1l** showed the highest bioavailability among those tested except the reference compound. The pivaloyloxymethyl esters of **1o** and **1s**, *i.e.* $\mathbf{8o}_1$ and $\mathbf{8s}_1$, were not absorbed orally at all.

In summary, among the cephalosporins prepared in this study, compound **1l** was the most promising candidate that might be further optimized both as a parenterally and orally available antibiotic effective against Gram-positive bacteria.

Acknowledgments

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