Studies on New Catechol Containing Cephalosporins

III. Synthesis and Structure-activity Relationships of Cephalosporins Having a Pyridone Moiety at the C-7 Position

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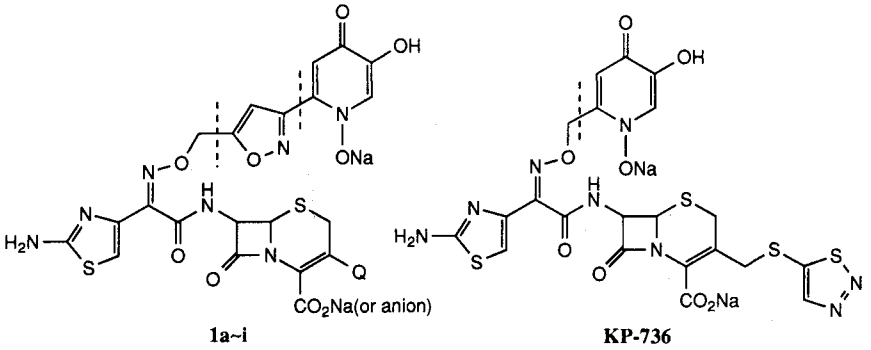
Recently, we reported the synthesis and antibacterial activity of cephalosporins containing a catechol moiety at C-3 and C-7 position, respectively[1,2]. As expected, they showed good activity especially against Gram-negative bacteria. There we found that the isoxazole spacer was essential for the enhancement of antibacterial activity against both Gram-positive and Gram-necgative strains. In recent years, cephalosporins have been reported bearing a mono- or dihydroxy pyridone instead of catechol moiety at C-7 side chain. This modification was found to improve the antipseudomonal activity and the stability to COMT, which had a good activity against Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*[3,4]. Therefore, we extended our search for cephalosporins with potent activity to those having 5-hydroxy-4-pyridone-*N*-oxide substituent in connection with the isoxazole spacer. Herein we wish to report the synthesis and *in vitro* antibacterial activity of **1h** and related compounds.

Chemistry

The synthesis of C-7 substituent having pyridone unit with an isoxazole spacer was depicted in Scheme 1. The pyridine *N*-oxide **2** which was prepared by known procedures[4], which primary alcohol was converted to an aldehyde by Swern oxidation and transformed into an isoxazole spacer according to the TAYLOR and RAY's method[5] to give the isoxazolylpyridine *N*-oxide **3**. The ester residue in the compound **3** was reduced by $NaBH_4$ and converted to the bromide **4** by triphenylphosphine-CBr_4. The key intermediate, alkoxylamine **5**, was obtained by following the Gabriel synthesis[6] from the bromide **4**, and finally condensed with protected aminothiazolylglyoxylic acid **6** to yield the desired C-7 substituent **7**. [7; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (3H, s, $PhOCH_3$), 5.13 (2H, s, CH_2), 5.30 (2H, s, OCH_2Ph), 6.45 (1H, s, isoxazol-H), 6.64 (1H, s, CH-thiazol), 6.85 (1H, s, $CHPh_2$), 7.00 (1H, s, pyridone-H), 7.10~7.80 (29H, m, Ph), 8.50 (1H, s, pyridone-H)].

The syntheses of new pyridone cephems **1a**~**1i** diverged at this point with regard to the C-3 substituents (Scheme 2). In case of the type I (**1a**~**1d**, Q=acetoxymethyl, chloro, vinyl or hydro), the introduction of C-3 substituent preceded the coupling of cephem moiety **8** and C-7 side chain **7**. On the other hand, the acylation step was followed by the introduction of C-3 substituent in the type II (**1e**~**1i**, Q=heterocyclylthiomethyl). Thus

Fig. 1.

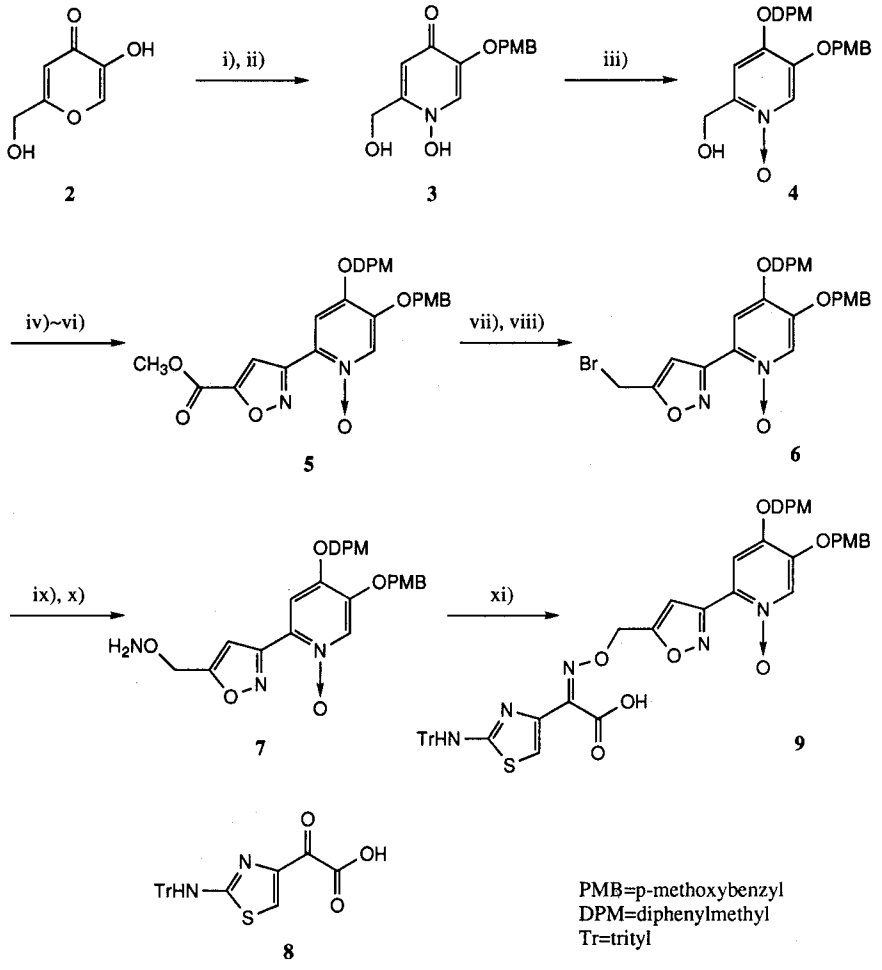


1a~i **KP-736**

1	a	b	c	d	e
Q	H	Cl	$HC=CH_2$	CH_2OAc	CH_2S–(N-N, N, CH_3)

Q	CH_2S–(N^+–R)	1	f	g	h	i
		R	Et	CH_2CH_2OH	$CH_2CO_2^-$	$NHCH_3$

Scheme 1. Synthesis of C-7 side chain.



Reagents i)PMB-Cl, K_2CO_3 ii)$H_2NOH{\cdot}HCl$, 41%(2steps) iii)Ph_2CN_2, Et_3N, 84% iv)Swern oxidation v)$H_2NOH{\cdot}HCl$ vi)NCS, Py; methyl propiolate, 48%(3 steps) vii)$NaBH_4$ viii)CBr_4, PPh_3, 64%(2 steps) ix)N-hydroxyphthalimide x)$H_2NNH_2{\cdot}H_2O$ 79%(2 steps) xi)**8**, 95%

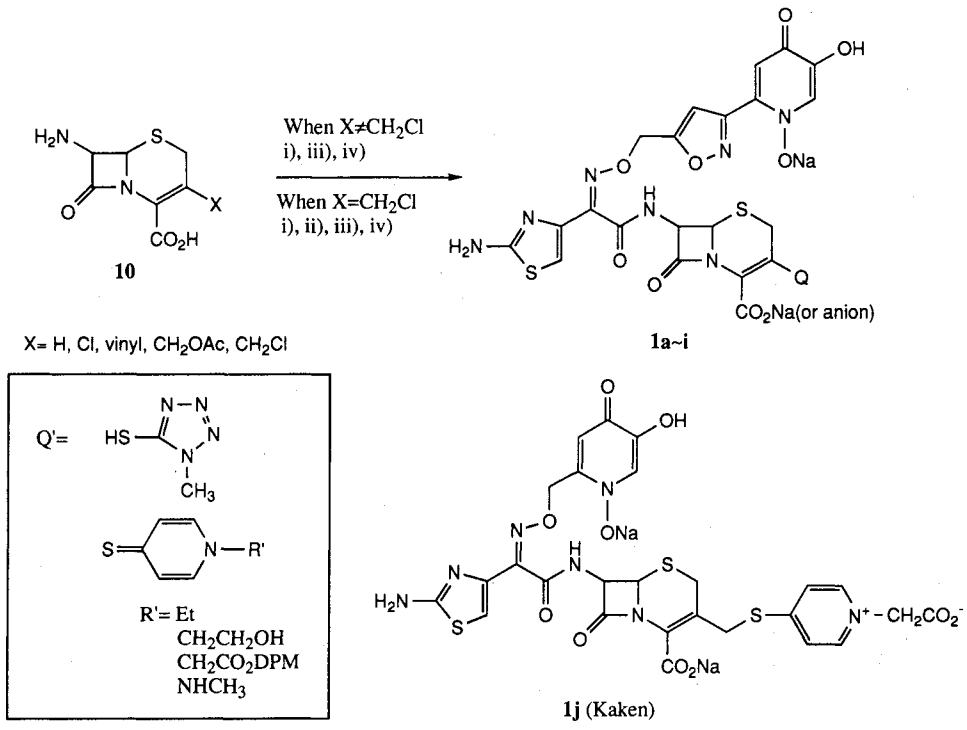
prepared protected cephalosporins were deprotected by using trifluoroacetic acid and anisole, and their sodium salts prepared by $NaHCO_3$ were subjected to chromatographic purification and lyophilization successively to give final products **1a**~**1i**, which were ready for biological evaluation. [**1h**; ^1H NMR (300 MHz, $CDCl_3$) δ 3.19~3.63 (2H, ABq, C_2-H), 4.13 (2H, ABq, CH_2S), 5.02 (2H, s, NCH_2), 5.12 (1H, d, C_6-H), 5.41 (2H, s, OCH_2), 5.74 (1H, d, C_7-H), 6.97 (1H, s, isoxazol-H), 7.04 (1H, s, CH-thiazol), 7.16 (1H, s, pyridone-H), 7.60 (1H, s, pyridone-H), 7.74 (2H, s, pyridine-H), 8.33 (2H, s, pyridine-H)] The compound **1j** which lacks only the isoxazole spacer from **1h** was also prepared to evaluate the effect of the spacer.

Biological Study

Tests of minimal inhibitory concentrations (MIC) of the new cephalosporins having 5-hydroxy-4-pyridone *N*-oxide moiety at C-7 position against both Gram-positive and Gram-negative strains were conducted and compared with cefotaxime and cefpirome, as controls, and the results are shown in Table 1. *In vitro* antibacterial activities of all the compounds prepared and controls were determined by the Mueller-Hinton agar dilution method.[7]

All the compound synthesized exhibited good activity against both Gram-positive and Gram-negative bacteria, particularly against *Pseudomonas aeruginosa*. The introduction of the pyridone moiety into C-7 position resulted in a significant enhancement of activity. It was apparent from the result that antibacterial activity of **1d** which was different only at C-7 side chain from cefotaxime was *ca.* 500~900 fold and 3~25 fold more potent than cefotaxime against *P. aeruginosa* and *E. coli*, respectively. However, activity of **1d** was reduced by factors of **2**~**8** against Gram-positive strains compared with

Scheme 2. Synthesis of cephalosporins **1a**~**i**.



Reagents i)$POCl_3$, Py, 51~96% ii)Q', NaI, 60~82% iii)TFA, Anisole iv)$NaHCO_3$, 51~81%

Table 1. *In vitro* antibacterial activity of cephalosporins **1a**~**i** (MIC, μg/ml).

Compound	*S. p.*	*E. f.*	*S. a.* 1	*S. a.* 2	*Es. c.* 1	*Es. c.* 2	*Es. c.* 3	*P. a.* 1	*P. a.* 2	*P. a.* 3	*S. t.*	*K. o.*	*En. c.* 1	*En. c.* 2
1a	0.098	>100	25	25	<0.002	<0.002	<0.002	0.049	0.049	0.013	<0.002	0.025	50	0.049
1b	0.025	>100	50	25	<0.002	<0.002	<0.002	0.013	0.20	0.007	<0.002	0.049	>100	0.098
1c	0.013	>100	6.25	6.25	<0.002	0.007	0.004	0.098	0.39	0.049	<0.002	0.025	50	0.098
1d	0.025	>100	12.5	6.25	<0.002	<0.002	0.004	0.025	0.025	0.007	<0.002	0.098	25	0.098
1e	0.025	>100	12.5	6.25	<0.002	<0.002	<0.002	0.013	0.013	0.007	<0.002	0.098	25	0.049
1f	0.007	>100	1.56	0.78	<0.002	<0.002	<0.002	0.098	0.098	0.049	<0.002	0.20	25	0.025
1g	0.098	>100	6.25	6.25	0.004	<0.002	0.004	0.39	0.098	0.098	<0.002	0.20	50	0.20
1h	0.098	>100	6.25	12.5	0.004	<0.002	0.004	0.025	0.013	0.013	<0.002	0.39	25	0.049
1i	0.049	100	1.56	3.13	<0.002	<0.002	<0.002	0.049	0.049	0.025	<0.002	0.39	50	0.049
1j	0.39	>100	25	50	0.007	<0.002	0.004	0.013	0.007	0.004	<0.002	0.098	12.5	0.20
Cefotaxime	0.004	100	1.56	3.13	0.049	0.007	0.025	12.5	12.5	6.25	0.025	0.78	100	0.004
Cefpirome	0.098	25	0.39	0.78	0.025	0.049	0.049	3.13	1.56	0.39	0.025	3.13	3.13	0.013

Abbreviations: *S. p.* = *Streptococcus pyogenes* 77A; *E. f.* = *Enterococcus faecium* MD8b; *S. a.* 1 = *Staphylococcus aureus* SG511; *S. a.* 2 = *Staphylococcus aureus* 285; *Es. c.* 1 = *Escherichia coli* SG511; *Es. c.* 2 = *Escherichia coli* DC2; *Es. c.* 3 = *Escherichia coli* TEM; *P. a.* 1 = *Pseudomonas aeruginosa* 9027; *P. a.* 2 = *Pseudomonas aeruginosa* 1592E; *P. a.* 3 = *Pseudomonas aeruginosa* 1771; *S. t.* = *Salomonella typhimurium*; *K. o.* = *Klebsiella oxytoca* 1082E; *En. c.* 1 = *Enterobacter cloacae* P99; *En. c.* 2 = *Enterobacter cloacae* 1321E.

cefotaxime. Activities of a series of new pyridone substituted compounds (**1a, d, e, f, h**) were increased about 2~12 fold against *E. coli* DC2 and *P. aeruginosa* 1771 than those of catechol type which we had reported in a previous publication[2]. But against *S. pyogenes* 77A and *S. aureus* SG511, they were less effective than the compounds possessing a catechol group by factors of 4~32. The effect of the isoxazole spacer could be measured by comparing the activities of **1h** with those of **1j** which lacks the isoxazole spacer. The compound **1h** gained 4 fold enhancement in antibacterial activities against *S. pyogenes* 77A and *S. aureus* SG511, but it showed a 2 fold reduced activity against *P. aeruginosa* compared to those of **1j**. The various substituents at C-3 position largely affected the activities of cephalosporins against Gram-positive strains. Among those bearing

Table 2. Pharmacokinetic parameters of new selected cephalosporins.

Route	**1h**		**1i**		Cefpirome	
	iv	im	iv	im	iv	im
C_{max} (μg/ml)	40.85±5.27	11.79±1.01	31.36±8.47	11.44±0.79	34.80±5.37	16.10±1.63
T_{max} (hr)	0.17	0.29±0.08	0.17	0.42±0.08	0.17	0.34±0.10
$T_{1/2}$ (hr)	0.51±0.04	0.71±0.05	0.60±0.00	0.65±0.03	0.64±0.14	0.61±0.14
AUC (μg·h/ml) (0~6 hr)	23.20±2.78	11.88±1.17	24.21±1.69	14.14±0.48	20.50±0.93	14.83±0.75
im/iv (%)	51.34±1.11		59±2.19		72.32±0.38	

Test microorganism: *S. pyogenes* 77A; Solvent: Saline; Animal: ICR mouse (25 g~30 g), 4 mice/compound/administration route; Dose: 40 mg/kg.

pyridinium group at C-3 position, **1f** and **1i** containing less polar group at pyridinium moiety than **1g** and **1h** showed better activity especially against *S. aureus*. In view of their antibacterial activities, **1h** and **1i** were selected for further evaluation. Pharmacokinetic parameters obtained *via* im and iv administration were shown in Table 2. The AUC values for both **1h** and **1i** were comparable to that for cefpirome. LD_{50} value for **1h** which was administered intravenously to 5 ICR mice of 18~19 g weight was >4000 (mg/kg).

Acknowledgments

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