Studies on New Catechol Containing Cephalosporins

I. Synthesis and Structure-activity Relationships of Cephalosporins Having a Catechol Moiety at the C-3 Position

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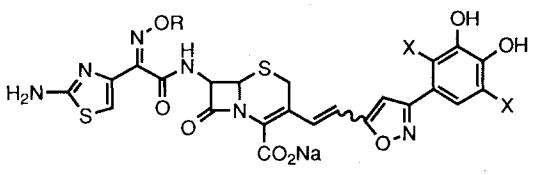
Many kinds of C-3 vinyl cephalosporins having various substituents at the vinyl group have been described. But few examples[1] could be found containing the "siderophore" at C-3 which facilitates the introduction of antibacterial agents into a bacterial cell by the iron-transport channel mechanism.[2] It was reported[3,4] that aminothiazolylcephalosporins bearing dihydroxy-aromatic moiety, *e.g.* catechol or 1,5-dihydroxy-4-pyridone, exhibit potent activity against Gram-negative bacteria including *Pseudomonas aeruginosa*. Thus we prepared 7-aminothiazolyl-3-vinylcephalosporins **1a**~**1j** having a catechol substituent linked to the vinyl group *via* an isoxazole moiety, which is known to enhance activity against Gram-positive bacteria, to achieve a balanced antibacterial spectrum and investigated their structure-activity relationships.

Fig. 1



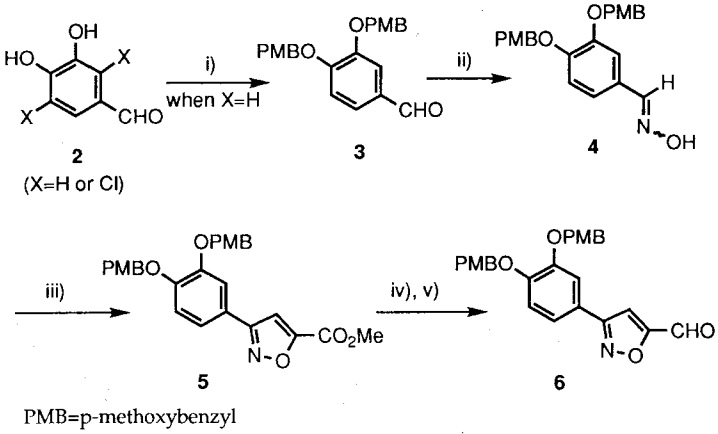
1a~j

X=H, Cl
R=H, Me, CH_2CO_2Na, $CH(S)(CH_3)CO_2Na$, $C(CH_3)_2CO_2Na$

Chemistry

Protection of 3,4-dihydroxybenzaldehyde (**2**, X=H) followed by oxime formation afforded aldoxime **4**, which was subjected to the 1,3-dipolar cycloaddition reaction according to TAYLOR and RAY'S protocol[5] (NCS, Py, methyl propiolate, Et_3N) to yield isoxazole **5**. The synthesis of aldehyde **6**, which can be coupled with the cephem nucleus by the Wittig reaction, was accomplished *via* the usual reduction-oxidation sequence (Scheme 1). (**6**; ^1H NMR (60 MHz, $CDCl_3$) δ 3.80 (6H, s, $2OCH_3$), 5.13 (4H, s, $2CH_2$), 6.90 (4H, d, 2Ph), 7.36 (4H, d, 2Ph), 7.01~7.63 (3H, m, catechol-H), 10.01 (1H, s, CHO)) After the reaction with phosphorus ylide **7** to give predominantly the (Z)-isomer (Z/E~9:1), the phenylacetyl group in the resulting cephalosporin **8** was cleaved (PCl_5, pyridine, MeOH) to yield 7-aminocephem **9**. 7-Aminocephem **9** was coupled (p-TsCl, Et_3N) with 2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetic acid (**10**) to give the protected cephalosporin **11**, which was then deprotected (TFA/anisole) and transformed into sodium salts **1a**~**1e**. Finally they were purified by reverse phase column chromatography (LiChrosorb RP-18, 30% aq methanol), and lyophilized. (**1b**; ^1H NMR (300 MHz, D_2O) δ 3.13~3.55 (2H, ABq, J=17.1 Hz, 2-H), 3.92 (3H, s, OCH_3), 5.27 (1H, d, J=4.5 Hz, 6-H), 5.78 (1H, d, J=4.5 Hz, 7-H), 6.42 (1H, d, J=12.0 Hz, 3-vinyl), 6.48 (1H, s, isoxazole-H), 6.70 (1H, d, J=12.0 Hz, 3-vinyl), 6.75 (1H, d, J=8.1 Hz, catechol-H), 6.91 (1H, s, thiazole-H), 7.02 (1H, d, J=8.1 Hz, catechol-H), 7.15 (1H, s, catechol-H)) Cephalosporins **1f**~**1j**, having a

Scheme 1. Synthesis of C-3 vinyl substituent.



PMB=p-methoxybenzyl

Reagents i)PMB-Cl, K_2CO_3, 80% ii)H_2NOH·HCl, Et_3N, 94% iii)N-chlorosuccinimide, Py, methyl propiolate, Et_3N, 49% iv)$NaBH_4$ v)Swern oxidation, 40%(2 steps)

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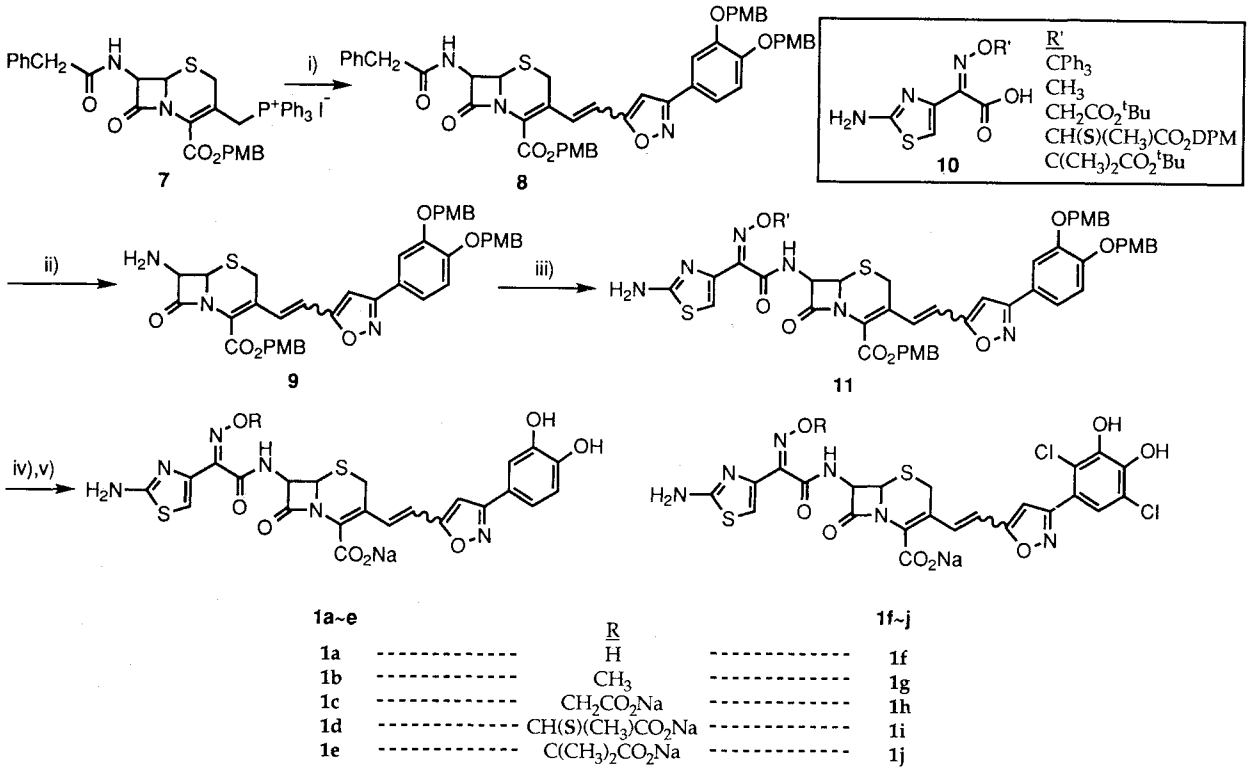
Scheme 2. Synthesis of new catechol type cephalosporins.



Reagents i)**6**, 5% $NaHCO_3$, CH_2Cl_2, 65%(Z/E=~9:1) ii)PCl_5, Py, 84% iii)**10**, pTsCl, Et_3N, 53~62% iv)TFA, Anisole v)7% $NaHCO_3$, 58~66% (2 steps)

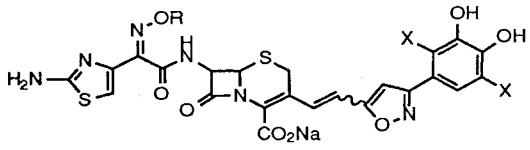
dichlorocatechol moiety which was known to improve the stability to catechol-*O*-methyl-transferase (COMT),[6] were synthesized from 2,5-dichloro-3,4-dihydroxybenzaldehyde (**2**, X=Cl) following the same synthetic pathway described above (Scheme 2). Cephalosporins **1k**[7] and **1l** were also synthesized for better understanding of the effect of structural modification.

Biological Studies

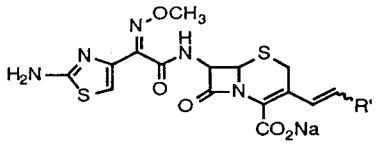
MICs of the new catechol type cephalosporins against both Gram-positive and Gram-negative strains were determined by the two-fold Mueller-Hinton agar dilution method,[8] and the results for selected strains are collected in Table 1. The MIC values of cefotaxime and cefpirome are also presented for comparison.

In Table 1, almost all the cephalosporins prepared showed balanced antibacterial activity against both Gram-positive and Gram-negative bacteria except against *S. faecium* which is known to be strongly resistant to cephalosporin family. Of the two series of cephalosporins, one bearing the unsubstituted catechol (**1a**~**1e**) showed more potent activity against Gram-positive bacteria than another with dichlorocatechol (**1f**~**1j**). Except **1a** and **1f** having hydroxyimino group at C-7 substituent, all the other cephalosporins exhibited antipseudomonal activity superior to both cefotaxime and cefpirome. And the compounds **1c**~**1e** and **1h**~**1j** possessing a carboxyl group on the C-7 side chain showed potent activity against Gram-negative strains, though they showed somewhat less activity against *E. cloacae*, possibly due to the polar nature of the carboxyl substituent. The catechol substituent enhanced the antipseudomonal activity as expected, which was also confirmed by comparing activity of **1k** and **1l**. However, the results against the other strains were unsatisfactory. The effect of isoxazole component could be measured by comparing the antibacterial activities of **1b** with those of **1l** which only lacks the isoxazole spacer. By simply adding the isoxazole moiety between vinyl and catechol substituents, **1b** gained large enhancement in activity against both Gram-positive and Gram-negative strains. This was confirmed by the results for the strains *S. pyogenes* and *S. typhimurium*, for which the increases in MIC values were *ca.* fifty- and one hundred-fold, respectively. Considering both potency and well-balanced spectrum of antibacterial activity, **1b** demonstrated optimal activity. It showed equivalent or superior activity to cefotaxime and cefpirome against all the strains tested except *E. cloacae*. The antibacterial activity of **1a** against Gram-positive bacteria, especially *S. faecium*, was excellent, but it showed poor activity against *K. oxytoca* and *E. cloacae*. Pharmacokinetic parameters obtained *via* im and iv administration of **1a**~**1e** are listed in Table 2. Large differences depending on the administration route were observed in the C_{max} and the AUC values, probably because of differing level of absorption, while the

Table 1. *In vitro* antibacterial activity of new catechol type cephalosporins (MIC, μg/ml).



1a~j



1k : R'=H 1l : R'=3,4-dihydroxyphenyl

Compound	X	R	*S. p.*	*S. f.*	*S. a.*	*Es. c.*	*P. a.*	*S. t.*	*K. o.*	*En. c.*
1a	H	H	0.003	6.25	0.39	0.006	3.13	0.012	>100	0.39
1b	H	CH_3	≤0.0015	25	1.56	≤0.0015	0.20	≤0.0015	6.25	0.098
1c	H	CH_2CO_2Na	0.049	>100	6.25	≤0.0015	0.049	0.006	0.39	0.098
1d	H	$CH(CH_3)CO_2Na$	0.20	>100	12.5	0.003	0.049	0.006	0.098	0.39
1e	H	$C(CH_3)_2CO_2Na$	0.098	>100	12.5	0.006	0.049	0.012	0.049	0.78
1f	Cl	H	0.20	>100	3.13	0.20	>100	0.39	3.13	12.5
1g	Cl	CH_3	0.025	100	6.25	0.003	0.39	0.003	50	0.39
1h	Cl	CH_2CO_2Na	0.20	100	12.5	0.006	0.098	0.006	0.78	0.78
1i	Cl	$CH(CH_3)CO_2Na$	0.20	100	12.5	≤0.0015	0.025	0.003	0.098	0.39
1j	Cl	$C(CH_3)_2CO_2Na$	0.39	>100	25	0.006	0.012	0.012	0.025	0.78
1k	-	-	0.003	>100	6.25	0.20	>100	0.20	1.56	0.20
1l	-	-	0.098	50	25	0.098	3.13	0.20	6.25	1.56
cefotaxime	-	-	0.003	100	1.56	0.006	12.5	0.025	0.78	0.003
cefpirome	-	-	0.098	25	0.39	0.049	1.56	0.025	3.13	0.012

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

Table 2. Pharmacokinetic parameters of new catechol type cephalosporins.

Parameters	Route	**1a**	**1b**	**1c**	**1d**	**1e**	Cefpirome
C_{max} (μg/ml)	iv	23.69±6.26	22.16±4.94	18.00±4.94	11.62±1.20	18.00±4.69	25.44±2.42
	im	13.90±2.22	9.38±0.77	9.64±0.74	7.20±0.37	8.12±1.14	21.66±3.09
T_{max} (hours)	iv	0.17±0.00	0.17±0.00	0.17±0.00	0.17±0.00	0.17±0.00	0.17±0.00
	im	0.17±0.00	0.17±0.00	0.17±0.00	0.17±0.00	0.17±0.00	0.33±0.00
$T_{1/2}$ (hours)	iv	1.07	0.76	1.24	0.97	0.75	0.66
	im	1.11	0.79	1.06	1.21	0.70	0.81
AUC (μg·hour/ml)	iv	23.57±5.07	23.73±7.90	16.95±2.69	11.23±1.42	13.07±4.69	22.79±2.42
	im	14.00±0.75	7.18±1.18	10.21±0.24	8.17±0.98	7.11±1.16	19.72±3.62
AUC im/iv (%)		59.3	30.3	60.2	72.7	54.3	86.5

Conditions: solvent=saline; medium=Mueller-Hinton agar; microorganism=*E. coli* 1507E; amount=40 (mg/kg); animal=female ICR mice, mean body weight=25.4 g, 4~6 mice per group.

reference compound cefpirome gave results irrespective of the administration route. The AUC values for both **1a** and **1b** *via* iv administration were comparable to that for cefpirome.

Acknowledgments

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