Synthesis and Antibacterial Activities of Novel C(7)-Catechol-substituted Cephalosporins (I)

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Cephalosporins have been widely used for treating diseases caused by pathogenic bacteria in human and animals. Through the *tonB* dependent iron transport mechanism,[1,2] the use of catechol substituted β-lactams was known to increase the drug's penetration into the bacterial cell walls. Thus, several catechol-substituted β-lactams have been synthesized and showed good anti-pseudomonal activities.[3~8] Despite their excellent anti-pseudomonal activities compared with those of other cephalosporins, most of the catechol substituted cephalosporins have weak activity against Gram-positive bacteria.[3~7] Also, there has been emerging resistance against cephalosporins, such as resistant *Escherichia coli* expressing TEM-like β-lactamases.

In the previous paper,[9] a new class of cephalosporins bearing 3-[(aminopyrimidiniumyl)thio] methyl substituents was found to exhibit well balanced activities against Gram-positive and Gram-negative bacteria. By introducing the cell penetration mechanism of the above catechol substituents on cephalosporins, while maintaining 3-[(aminopyrimidiniumyl)thio] methyl substituents on cephem nucleus, we wish to synthesize new cephalosporins which have not only broader spectrum of antibacterial activities including *Pseudomonas aeruginosa*, but also increased stabilities against various β-lactamases and longer plasma half life. Thus, we have prepared a series of novel compounds which possess both catechol moiety at the 7-position and pyrimidiniumthio methyl, pyrimidinothio methyl or pyrimidinium methyl group at the 3-position of cephem nucleus (Fig. 1). We report herein the synthesis of these compounds and their antimicrobial activities.

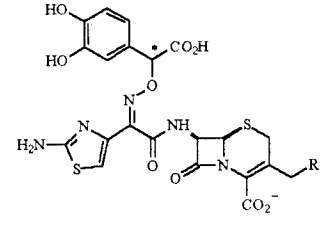
Chemistry

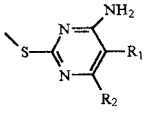
The compounds **1**~**10** from oxime **11** and bromide **12** were prepared as follows (Scheme 1). Oxime **11** and benzyl bromide **12** was coupled in the presence of potassium carbonate and potassium iodide in *N,N*-dimethylformamide (DMF) to afford the oxyimino compound **13**. Removal of the allyl moiety on the compound **13** was performed by using Pd(0) catalyzed reaction to give the carboxylic acid **14**. The acid **14** was added to the methylene chloride solution containing *p*-methoxybenzyl-7-amino-3-chloromethyl-3-cephem-4-carboxylate (7-ACLE) and pyridine at $-20°C$, then phosphorous oxychloride ($POCl_3$) was added to the solution to afford the coupling product **15**. Then the chloride **15** was displaced with pyrimidine thiones or pyrimidines in DMF and deprotection of the corresponding coupling products in the presence of trifluoroacetic acid (TFA) and anisole afforded the cephalosporins **1***S*,*R*~**10***S*,*R*† (Most compounds have two diastereomers which contain *R* and *S* configuration on the benzylic position of catechol moiety. Thus, from now on, each diastereomers will be described as *R* and *S*). Spectra for **1***S*: IR (nujol) 1770 cm^{-1} (carbonyl on β-lactam ring); ^1H NMR (δ, D_2O) 3.29 (ABq, 2H, J=15.1 Hz), 4.07 (ABq, 2H, J=13.5 Hz), 4.96 (d, 1H, J=2.5 Hz), 5.37 (s, 1H), 5.42 (s, 1H), 5.62 (d, 1H, J=2.5 Hz), 6.81 (d, 1H, J=7.8 Hz), 6.91 (d, 1H, J=7.8 Hz), 7.01 (s, 2H).

Antibacterial Activities and Discussion

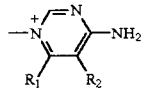
Agar dilution method was used to determine the minimal inhibitory concentration (MIC) of compounds **1**~**10** against selected organisms. The MIC values for ceftazidime against the same strains are shown for comparison. In general, most of the compounds in Table 1 showed better antibacterial activities than those of the

Fig. 1. Novel catechol-substituted cephalosporins **1**~**10** with various C-3 substituents.

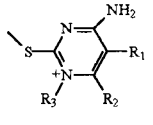




No.	R_1	R_2
1*R*,*S*	H	NH_2
2*R*,*S*	Me	NH_2
3*S*	H	H



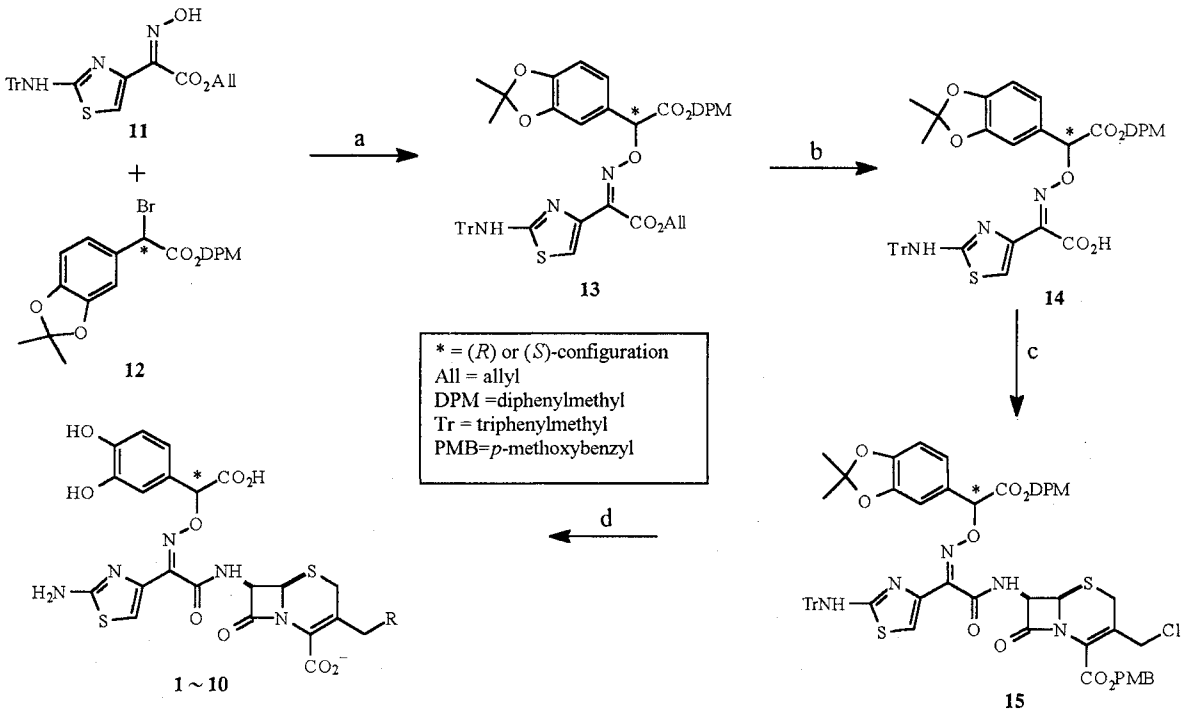
No.	R_1	R_2
4*R*,*S*	H	H
5*R*,*S*	$-CH_2CH_2CH_2-$	



No.	R_1	R_2	R_3
6*R*,*S*	H	H	Me
7*R*,*S*	H	H	NH_2
8*R*,*S*	H	NH_2	Me
9*R*,*S*	Me	NH_2	NH_2
10*R*,*S*	$-CH_2CH_2CH_2-$		NH_2

† *R* and *S* configurations were proved by the method shown in the ref. 10.

Scheme 1.



a) K_2CO_3, KI, DMF; b) $Pd(PPh_3)_4$, potassium 2-ethylhexanoate; c) $POCl_3$, 7-ACLE; d) (1)Nucleophile (R); (2) TFA, Anisole.

Table 1. Antibacterial activities of cephalosporins **1～10** (MIC, μg/ml).

Compound	*S.a.*	*E.f.*	*E.c.*1	*E.c.*2	*P.a.*	*A.c.*	*E.c.*	*K.a.*	*S.m.*
1*S*	2	>128	<0.008	0.031	0.25	0.13	16	0.13	0.25
2*S*	2	>128	<0.008	0.031	0.25	0.5	8	0.13	0.25
3*S*	4	>128	0.063	0.13	1	2	128	0.5	1
4*S*	0.5	32	0.13	0.13	2	1	64	1	0.5
5*S*	0.5	4	0.063	0.13	1	1	16	1	0.25
6*S*	0.5	64	0.031	0.063	0.5	0.5	16	0.25	0.5
7*S*	1	64	0.016	0.063	0.25	0.25	8	0.13	0.25
8*S*	0.5	32	0.063	0.063	0.5	0.5	16	0.25	0.5
9*S*	0.25	32	0.031	0.063	0.5	0.5	8	0.13	0.25
10*S*	0.5	16	0.031	0.063	0.5	0.25	2	0.13	0.5
Ceftazidime	16	>128	0.13	0.25	1	2	64	0.25	0.25

S.a., *Staphylococcus aureus* ATCC-6538p; *E.f.*, *Enterococcus faecalis* 29212; *E.c.*1, *Escherichia coli* ATCC-10536; *E.c.*2, *Escherichia coli* TEM1 1193E; *P.a.*, *Pseudomonas aeruginosa* 1912E; *A.c.*, *Acinetobacter calcoaceticus* 15473; *E.c.*, *Enterobacter cloacae* P99; *K.a.*, *Klebsiella aerogenes* SHV-1 1976E; *S.m.*, *Serratia marcescens* 1826E.

Table 2. Pharmacokinetic data of the compounds **1～4**, **6～10** in rats.

Parameters	**1***S*	**2***S*	**3***S*	**4***S*	**6***S*	**7***S*	**8***S*	**9***S*	**10***S*	CAZ
$T_{1/2}$ (minute)	62	55	58	59	39	50	53	40	53	20
AUC (μg·minute/ml)	3694	3247	3472	3571	2614	3174	3324	3152	3868	1863

reference (CAZ: ceftazidime). This series of new catechol substituted cephalosporins exhibited good antibacterial activities against Gram-positive bacteria such as *S. aureus* and excellent activities against Gram-negative organisms including *Pseudomonas aeruginosa*. The cephalosporins **1**~**10** showed better antibacterial activities against most of the Gram-positive strains than the other catechol-containing cephalosporins.[3~8] Interestingly, most cephalosporins shown in Table 1 displayed poor activity against *E. faecalis*. It is worthwhile to note that the compounds having (*S*)-configuration on the catechol side chain were significantly more active than the compound which contains the corresponding (*R*)-side chain (The MIC's of (*R*)-isomers are not shown in this paper but in paptents).[11] Cephalosporins with pyrimidiniumyl substituents **4**, **5** exhibited similar potency against Gram positive bacteria to the compounds substituted by thiopyrimidiniumyl **6**~**10** and thiopyrimidinyl derivatives **1**~**3**, but showed poor activity against Gram-negative strains, especially against *P. aeruginosa*. Among these series of compounds, cephalosporins with thiopyrimidiniumyl moiety exhibited the most balanced antibacterial activity profiles.

As expected by the mechanism described before, cephalosporins **1**~**10** had a good potency against *P. aeruginosa*, especially the (*S*)-configurated diastereomers showed excellent anti-pseudomonal activities. The compounds **1**~**10** were also very stable to the extended spectrum of TEM-like β-lactamases. Thus, they possessed very good activities against resistant *E. coli* which express TEM 1 β-lactamases. They also displayed better antibacterial activities against *K. aerogenes* expressing SHV-1 β-lactamase than that of the ceftazidime, but gave comparable activities against *E. cloaceae* expressing P99 β-lactamase compared to CAZ. Pharmacokinetic studies on the new cephalosporins **1**~**3**, **6**, **7**~**10** which have pyrimidinyl- and pyrimidinium-thiomethyl substituents were shown in Table 2. In rats, they showed significantly higher AUC values and longer half life compared to ceftazidime after a dose of 20 mg/kg intravenously.

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