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SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NOVEL CLASS OF CEPHALOSPORINS WITH A OXADIAZOLYL HYDROXYPYRIDONE MOIETY AT C-7

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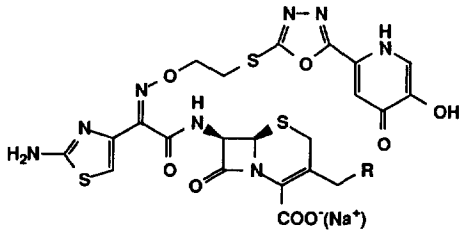
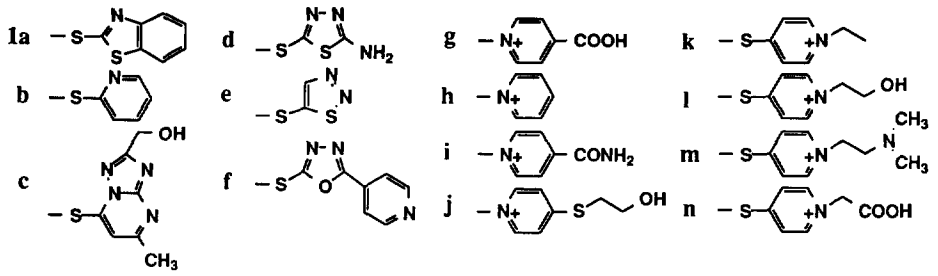
Abstract Synthesis and biological properties of a novel class of cephalosporins with a 2-[5-(3-hydroxy-4-pyridon-6-yl)1,3,4-oxadiazol-2-yl]thioethyl group at C-7 position are described. Among them, the compounds having a pyridiniumthiomethyl group at C-3 position were found to possess high *in vitro* potency and showed excellent *in vivo* efficacy against both *S. aureus* and *P. aeruginosa*.

The nosocomial infections caused by various Gram-negative bacteria including *Pseudomonas aeruginosa* and *Staphylococcus aureus* including MRSA, have progressively increased and become a serious problem in chemotherapy[1]. It has been demonstrated that introduction of a catechol group or its bioisoster into cephalosporins enhanced *in vitro* potency against Gram-negative bacteria including *P. aeruginosa*[2]. This enhanced antibacterial activity has been concerned to be due to the ability of penetrating to the outer membrane of organisms such as *Escherichia coli via ton B*-dependent iron transport pathway[3]. Recent reports presented that





a few cephalosporin with catechol exhibited good anti-pseudomonal *in vivo* efficacy[4]. However, most of the catecholic cephalosporins were shown to be ineffective for Gram-positive bacteria especially *S. aureus* and unsatisfactory to *in vivo* efficacy not only against the Gram-positive bacteria but also against the Gram-negative bacteria. Such characteristics seems to prevent them showing sufficient therapeutic efficacy. Therefore, a study of creation of novel cephalosporins possessing good antibacterial activity (both *S. aureus* and *P. aeruginosa*) and good *in vivo* efficacy was worthwhile in cephalosporin chemistry. Thus, our efforts have been focused on synthesizing a novel hydroxypyridone group at C-7 position of cephalosporin with enhanced antibacterial activity and improved *in vivo* efficacy. As a result, we have discovered the novel substituent of C-7 side chain, (Z)-2-(2-aminothiazol-4-yl)-2-[2-(5-(3-hydroxy-4-pyridon-6-yl)1,3,4-oxadiazol-2-yl)thio]ethoxyiminoacetamido group. In this communication, we wish to describe the synthesis of cephalosporins having a novel hydroxypyridone group at C-7 position and their biological effects.

Synthesis

The synthesis of novel hydroxypyridone cephalosporins was outlined in Scheme.

First, protected hydroxypyridone derivative **6** which is the key intermediate in the synthesis of novel cephalosporins was prepared. Carboxylic acid **3** was prepared from corresponding alcohol **2**[5] by two steps oxidation in 94% yield. Esterification and O-protection were carried out in the compound **3** at the same time to afford **4** which was easily converted to hydrazide **5** in an overall yield of 78% from **3**. Compound **5** was cyclized with carbon disulfide to afford 1,3,4-oxadiazol derivative **6** in 99% yield.

Second, construction of iminoacetic acid **10** from **6** and introduced to C-7 position of cephalosporin were achieved.

N-hydroxyphthalimide was treated with excess amount of 1,2-dibromoethane, followed by introduction of compound **6** to gave **8** in 60% yield. After deprotection of phthaloyl group, the resulting alkoxyamine **9** was coupled with glyoxylic acid to afford novel iminoacetic acid **10**[6] in 90% yield from **8**. An amino group of ACLE was acylated with **10** in the presence of DCC to furnish compound **11** in 71% yield.

In the finally, after treatment of **11** with various nucleophile, all protecting groups were removed with TFA in the presence of anisole to give desired novel cephalosporins **1a~n** in 21~62% yield[7].

Biological properties

Compounds **1a~n** were evaluated for *in vitro* antibacterial activities against 4 organisms[8]. In Table 1, their minimum inhibitory concentrations(MICs) are summarized, ceftazidime(CAZ) and cefotaxime(CTX) as reference compounds is also presented[9].

On the whole, there was no significant difference in *in vitro* activities against all organisms, except the containing carboxylic group in the C-3 side chain(**1g** and **1n**) which was inferior to the other compounds against *S. aureus*. The heterocyclicthio group(**1a~f**) and pyridiniumthio group(**1k~m**) showed more broad antibacterial spectrum and higher antibacterial activity than the pyridinium group(**1h** and **1i**). Especially, compound **1c~e**, **1k** and **1l** were much more active against two strains of *P. aeruginosa* than CAZ.

Scheme. Synthesis of novel cephalosporins.

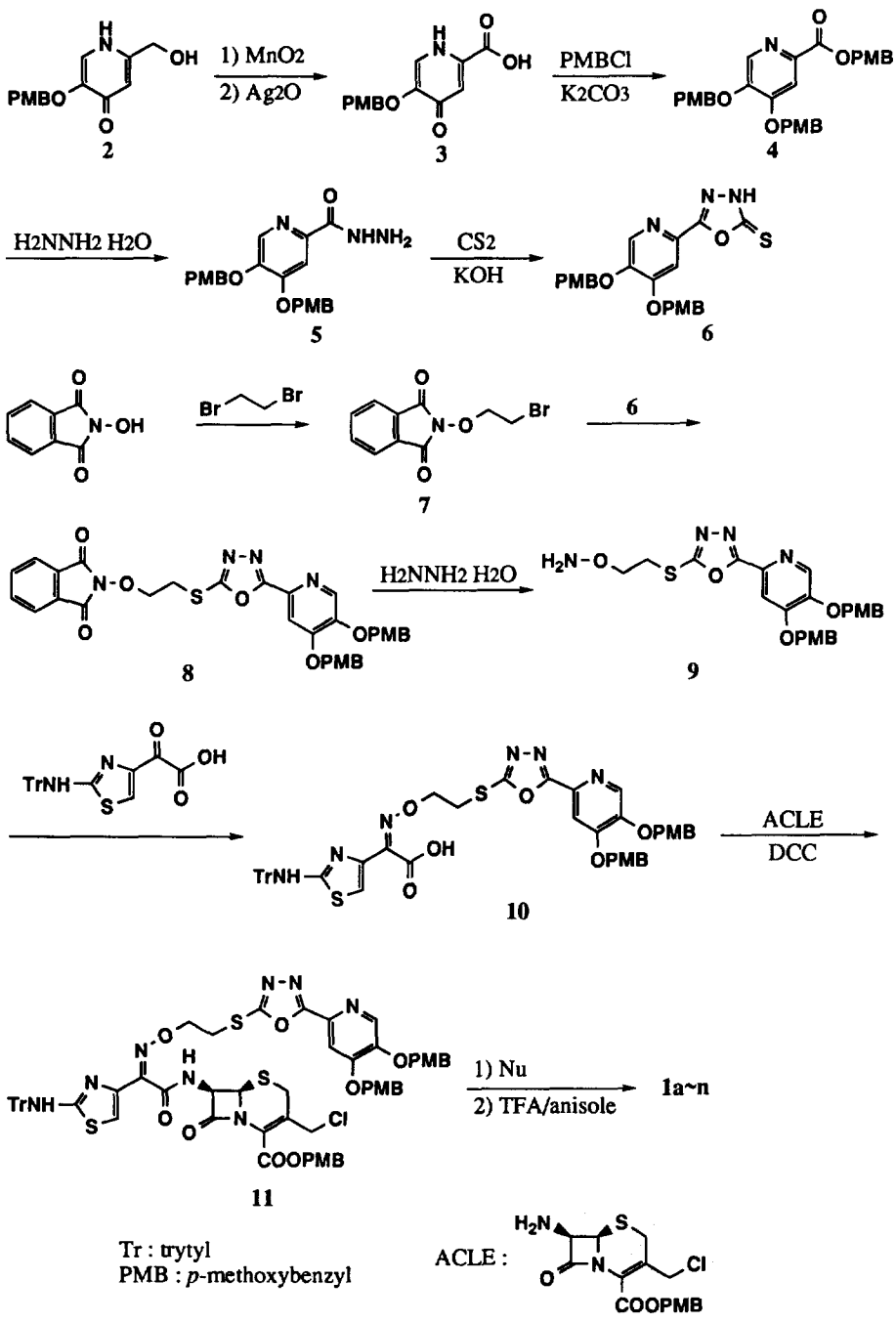


Table 1. Antibacterial activity (MICs, μg/ml) of novel cephlosporins.

Organism	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**
S. a. Smith	3.13	6.25	6.25	6.25	3.13	6.25	25
E. c. ML4707	0.0125	≤0.0063	<0.0063	≤0.0063	≤0.0063	≤0.0063	0.0125
P. a. E-2	0.78	0.39	0.20	0.20	0.78	0.78	3.13
P. a. IFO12689	0.78	0.39	0.39	0.20	0.20	0.39	0.39

Table 1. (Continued.)

Organism	**1h**	**1i**	**1j**	**1k**	**1l**	**1m**	**1n**
S. a. Smith	3.13	6.25	3.13	3.13	3.13	6.25	12.5
E. c. ML4707	0.025	0.025	0.0125	≤0.0063	≤0.0063	0.025	≤0.0063
P. a. E-2	3.13	3.13	0.78	0.39	0.39	0.78	1.56
P. a. IFO12689	0.78	1.56	0.78	0.78	0.78	3.13	0.39

Table 1. (Continued.)

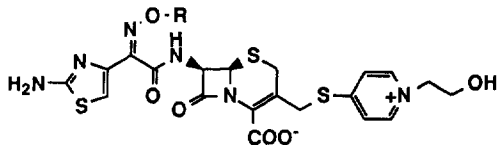
Organism	CAZ	CTX
S. a. Smith	3.13	1.56
E. c. ML4707	0.05	0.0125
P. a. E-2	1.56	25
P. a. IFO12689	3.13	25

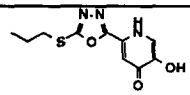
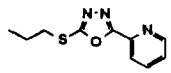
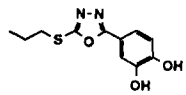
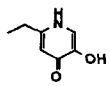
Abbreviations:
S. a., Staphylococcus aureus;
E. c., Escherichia coli;
P. a., Pseudomonas aeruginosa.

Table 2. Therapeutic efficacy of novel cephalosporins in systemic infections in mice.

Test organism	Challenge dose (cfu/mouse)	Compounds	MIC(mg/ml)	ED50(mg/kg)	95% confidence limits (mg/kg)
P. aeruginosa E-2	8.0×10^4	**1a**	0.78	>100	
	(+5% mucin)	**1d**	0.20	35	15 - 82
		1k	0.39	19	9.4 - 39
		1l	0.39	4.2	2.6 - 6.7
		1n	1.56	25	11 - 55
		CAZ	1.56	55	29 - 103
S. aureus Smith	5.0×10^6	**1d**	6.25	>20	
	(+5% mucin)	**1k**	3.13	<0.6	
		1l	3.13	<0.3	
		CAZ	3.13	8.1	4.3 - 15
		CTX	1.56	4.6	3.1 - 6.9

Table 3. Antibacterial activity and protective effects of **1l** in comparison with related compounds.



	Compounds (R)	*S.a.* Smith MIC(mg/ml)	*S.a.* Smith ED50(mg/kg)*	*P. a.* E-2 MIC(mg/ml)	*P. a.* E-2 ED50(mg/kg)**
1l		3.13	0.86 (0.43 - 1.7)	0.39	7.2 (3.3 - 16)
1p		0.39	0.43 (0.25 - 0.74)	100	>40
1o		0.39	0.86 (0.51 - 1.4)	0.39	34 (19 - 60)
1q		3.13	0.86 (0.51 - 1.4)	6.25	>40
	CAZ		N.T.	1.56	>100
	CTX	1.56	3.0 (2.3 - 4.0)		N.T.

Abbreviations: See footnote in Table 1; N.T. : not tested.
* 3.3×10^6(cfu/mouse, +5% mucin), ** 7.9×10^4(cfu/mouse, +5% mucin).
95% confidence limits(mg/kg) are designated in parentheses.

The protective effects of selected compounds, CAZ and CTX were examined on systemic infection in mice[10]. The results are shown in Table 2. Except the benzothiazole derivative **1a**, most of the other selected cephalosporins showed better *in vivo* activity against *P. aeruginosa* as well as *in vitro* activity than CAZ. Against *S. aureus*, **1k** and **1l** exhibited much more excellent therapeutic efficacy than CTX and CAZ. Among the all compounds, **1l** showed the best efficacy against both *S. aureus* and *P. aeruginosa*, which was more than 10-fold potent, compared with those of CTX and CAZ.

A comparison of antibacterial activities between a variety of the related substituents on oxime moiety was indicated in Table 3[11]. All listed compounds showed better *in vivo* activity against *S. aureus* than CTX. As we expected, simple pyridine substituted compound **1p** was inactive against *P. aeruginosa in vivo* also *in vitro*. It was noteworthy that, in spite of having a catechol group or hydroxypyridone group, anti-pseudomonal *in vivo* activity of compound **1o** or **1q** was inferior to that of **1l**. Especially, compound **1q** was inactive against *P. aeruginosa*. It was characteristic that **1q** was devoid of 1,3,4-oxadiazole-2-thio group.

Based these results, the 2-[5-(3-hydroxy-4-pyridon-6-yl)1,3,4-oxadiazol-2-yl]thioethyl group as a novel substituent at C-7 position not only display the potent antibacterial activity but also contributes to the excellent

therapeutic efficacy against both strain *S. aureus* and *P. aeruginosa*. In particular, the protective effect against *S. aureus* Smith of **11** was superior to that of cefpirome[9] (**11**, ED50: 0.96mg/kg and cefpirome, ED50: 1.5mg/kg)[12]. Further studies on these novel cephalosporins and the further functional evaluation of a 2-[5-(3-hydroxy-4-pyridon-6-yl)1,3,4-oxadiazol-2-yl]thioethyl group are in progress.

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References and Notes

1 a) Kiehn, T. E. ; *Eur. J. Clin. Microbiol. Infect. Dis.*, **1989**, *8*, 832. b) Kykyn, S. J. ; *Lancent*, **1988**, *8577*, 100.

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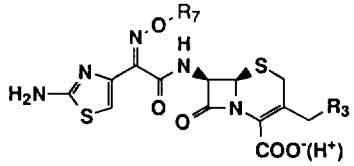
6 **10**: ^{1}H-NMR(400MHz, DMSO-*d*6)δ: 3.51(2H, t, *J*=5.9Hz), 3.746(3H, s), 3.751(3H, s), 4.23(2H, t, *J*=5.9Hz), 5.21(2H, s), 5.25(2H, s), 6.61(1H, s), 6.94(2H, d, *J*=9.8Hz), 6.96(2H, d, *J*=8.8Hz), 7.21~7.41(19H, m), 7.80(1H, s), 8.43(1H, s), 8.62(1H, s).

7 **11**: ^{1}H-NMR(400MHz, D2O+DMSO-*d*6)δ: 3.565(1H, d, *J*=18.6Hz), 3.571(2H, t, *J*=6.8Hz), 3.75(1H, d, *J*=18.6Hz), 3.80(2H, t, *J*=5.4Hz), 4.33~4.49(6H, m), 5.21(1H, d, *J*=4.9Hz), 5.83(1H, d, *J*=4.9Hz), 6.80(1H, s), 7.49(1H, s), 7.99(2H, d, *J*=6.8Hz), 8.07(1H, s), 8.67(2H, d, *J*=6.8Hz). IR(KBr, cm^{-1}): 3200, 1760, 1600, 1620, 1540, 1470. SIMS(positive, m/z): 774[M+H]$^+$.

8 Japan Society of Chemotherapy, *Chemotherapy*, **1981**, *29*, 76.

9 The chemical structure of reference compounds.



ceftazidime: R3= –N⁺⟨ ⟩ , R7=C(Me)2COOH

cefpirome : R3= –N⁺⟨ ⟩ , R7=Me

cefotaxime : R3=OCOCH3, R7=Me

10 The test compounds were administered subcutaneously one hour after challenged. Number of mice untreated and treated groups at each dose was 5 (n=5). The 50% effective dose(ED50) were calculated by the Litchfield-Wilcoxon method on the basis of the number of survivors at 7 days after infection.

11 **1p**, **1o** and **1q** were synthesized in a similar manner as **11**.

12 Inoculum size: 5.5x10^6 cfu/mous+5%mucin. 95% confidence limits,**11**: 0.49 - 1.9mg/kg, cefpirome: 1.0 - 2.2mg/kg.

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