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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CEPHALOSPORINS HAVING C-3 CATECHOL-CONTAINING (PYRIDINIUM-4'-THIO)-METHYL GROUPS

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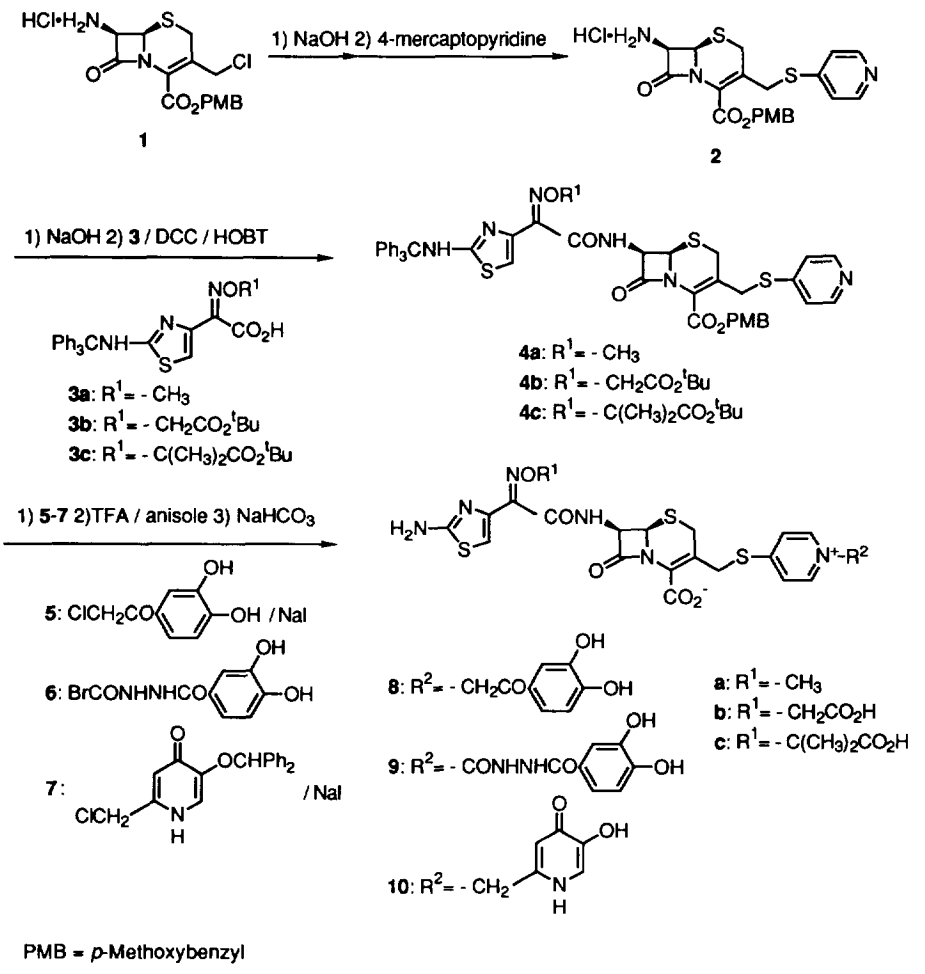
Abstract: Cephalosporins with catechol-containing (pyridinium-4'-thio)methyl groups at C-3 have been synthesized and examined their antibacterial activity. They were found to have high potency against gram-negative bacteria including *Pseudomonas aeruginosa*. In particular, compound **9c** bearing {1'-[*N*-(3",4"-dihydroxybenzamide)carbamoylmethyl]pyridinium-4'-thio}methyl group at C-3 exhibited excellent anti-pseudomonal *in vivo* efficacy.

In the course of our ongoing research for more potent antibiotics against Methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas aeruginosa* which had low sensitivity toward almost clinically used anti-infectives, we have found that 3-(*N*-alkylpyridinium-4'-thio)methyl-2-oxaisocephems exhibited excellent *in vitro* and *in vivo* activity against gram-positive bacteria including MRSA,[1] and 2-isocephems bearing a 1,3-dihydroxy-4-pyridone group which was the so-called catechol related aromatics, at C-7 possessed potent *in vitro* activity and good protective effects on systemic infection in mice against gram-negative bacteria including *Pseudomonas aeruginosa*.[2] Therefore, we took interest in biological activity of cephalosporins and their analogues which had both 3-(*N*-alkylpyridinium-4'-thio)methyl[3] and catechol group or related aromatics because these compounds could be expected to possess broad spectrum and potent activity. In this communication, we wish to describe the synthesis and antimicrobial activity of cephalosporins bearing C-3 catechol-containing (pyridinium-4'-thio)methyl groups.

Synthesis

The required cephalosporins were synthesized by the process depicted in Scheme 1. *p*-Methoxybenzyl (6*R*, 7*R*)-7-amino-3-chloromethyl-3-cephem-4-carboxylate hydrochloride **1**[4] was neutralized with aqueous solution of NaOH and treated with 4-mercaptopyridine in CH_2Cl_2 at r.t. to afford 3-(pyridinium-4'-thio)methyl derivative monohydrochloride **2** in 93 % yield. After libration of amino group, **2** was condensed with active esters derived from 2-triphenylmethylaminothiazoles **3a-c**[5] and 1-hydroxybenzotriazole (HOBT) in CH_2Cl_2 at r.t. to furnish

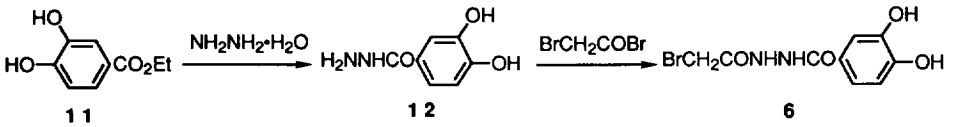
Scheme 1



compounds **4a-c** in 17-57 % yield. Thus obtained **4a-c** were converted to the desired compounds **8-10** as follows; compounds **4a-c** were quaternarized by alkyl halides **5-7** bearing catechol or 3-hydroxy-4-pyridone moiety[6] in DMF at r.t., and deprotection was achieved by TFA in the presence of anisole at 0℃. The resulting TFA salts were neutralized by $NaHCO_3$ and purified by Diaion HP-20 column chromatography to afford **8-10** in 3-59 % yield from **4a-c**.

2-Bromo-[*N*-(3',4'-dihydroxybenzamidoyl)]acetamide **6** was prepared as illustrated in Scheme 2. Ethyl 3,4-dihydroxybenzoate **11** was treated with hydrazine monohydrate without solvent at r.t. to give hydrazide **12** in 93 % yield. Compound **12** was condensed with bromoacetyl bromide in dioxane at r.t. to afford required acetamide **6** in 40 % yield.

Scheme 2



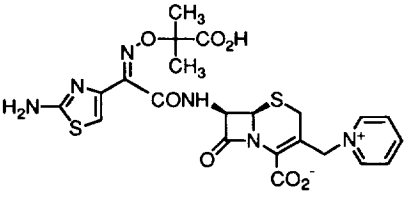
11 12 6

Biological Assays

The minimum inhibitory concentrations (MICs) of the cephalosporins with C-3 catechol containing (pyridinium-4'-thio)methyl groups (**8a-c**, **9a-c**, and **10c**) were tested by using a two-fold agar dilution method.[7] In Table 1, the MIC values of these compounds against several gram-positive (*Staphylococcus aureus* FDA 209P) and gram-negative bacteria (*Escherichia coli* NIHJ JC-2, *P. aeruginosa* ATCC-10145, and *P. aeruginosa* NCTC-10490) are shown and compared with those of ceftazidime (CAZ). New cephalosporins **8a-c**, **9a-c**, and **10c** exhibited more potent activity against gram-negative bacteria including *P. aeruginosa* than CAZ. Especially, compounds **8b**, **8c**, **9b**, and **9c** bearing a carboxyl group at C-7 possessed the best activity against *P. aeruginosa*, while their activity against *S. aureus* were inferior to compounds **8a** and **9a** with C-7 methoxyimino moiety. In comparison of activity of compounds **8c**, **9c**, and **10c**, compound **10c** bering 3-hydroxy-4-pyridone moiety at C-3 was less active than others.

Table 1. *In vitro* Antibacterial Activity [MICs (μg/ml), Inoculum size: 10^6 cells/ml]

Compd.	*S. aureus* FDA 209P	*E. coli* NIHJ JC-2	*P. aeruginosa* ATCC-10145	*P. aeruginosa* NCTC-10490
8a	0.78	0.1	0.39	0.05
8b	3.13	0.05	0.1	≤0.025
8c	3.13	0.1	0.05	≤0.025
9a	0.78	0.05	0.78	0.05
9b	3.13	≤0.025	0.1	≤0.025
9c	3.13	≤0.025	0.1	≤0.025
10c	6.25	0.78	0.39	0.05
CAZ	3.13	0.39	1.56	0.78



ceftazidime (CAZ)

The *in vivo* activity of the selected compounds **8b**, **9b**, **9c**, and CAZ against systemic infections with *P.*

aeruginosa E-2 in mice are listed in Table 2. One hour after intraperitoneal infection, several doses of each compound were subcutaneously administered to mice. Fifty percent effective dose values (ED_{50}) (mg / kg) were caluclated by the probit method from the number of mice surviving 7 days after infection.

Table 2. Therapeutic efficacy against systemic infections in mice

Test organism	Compounds	MIC (μg/ml)	Challenge dose (cells/mouse)	ED_{50} (mg/kg)	95 % Confidence limits (mg/kg)
P. aeruginosa E-2	**8b**	0.1	1.0×10^6	>20	
	9b	0.2	1.0×10^6	>25	
	9c	0.1	1.0×10^6	10.19	5.83-19.78
	CAZ	1.56	1.0×10^6	34.44	16.85-53.84

Although compounds **8b** and **9b** were inactive in mice against an experimental infection with *P. aeruginosa* E-2, compound **9c** exhibited better therapeutic efficacy than CAZ.

In conclusion, we have found that cephalosporins with catechol-containing (pyridinium-4'-thio)methyl groups at C-3 showed potent anti-pseudomonal activity and broad spectrum, besides compound **9c** possessed better *in vivo* efficacy on systemic infection in mice caused by *P. aeruginosa* E-2 than CAZ did.

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