Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 13, pp. 1601-1606, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00209-6

SYNTHESIS AND ANTI-PSEUDOMONAL ACTIVITY OF NEW 2-ISOCEPHEMS WITH A DIHYDROXYPYRIDONE MOIETY AT C-7

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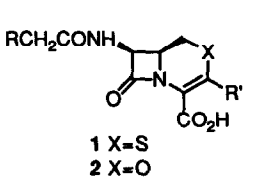
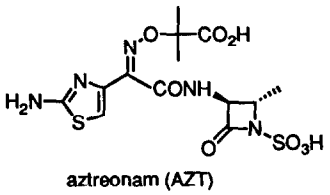
Abstract: The synthesis and biological activities of optically active new 2-isocephems with a 1,3-dihydroxy-4-pyridone moiety at C-7 are described. They were found to have potent antibacterial activity against gram-negative bacteria including *Pseudomonas aeruginosa*. Among them, 3-[(4-methyl-5-carboxymethyl)thiazol-2-yl]thiomethyl derivative **10d** possessed excellent anti-pseudomonal *in vitro* potency and *in vivo* efficacy.

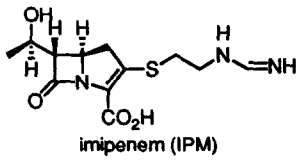
Nuclear analogues of cephalosporins, such as 2-isocephems **1** and 2-oxaisocephems **2**, have been prepared and some of them known to have potent antibacterial activity.[1] Previously, we prepared optically active new 2-oxaisocephems with thio-substituted methyl groups at C-3 and 2-aminothiazol-4-yl moiety at C-7 for the sake of enhancement of antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) which sometimes causes lethal nosocomial infection.[2] On the other hand, the opportunistic infections attributed to miscellaneous gram-negative pathogens have come to be a serious problem in chemotherapy. In particular, some strains of *Pseudomonas aeruginosa* show resistance to clinically used anti-pseudomonal agents, ceftazidime (CAZ), aztreonam (AZT), and imipenem (IPM), so that the search and development of new agents is required.



1 X=S
2 X=O



aztreonam (AZT)



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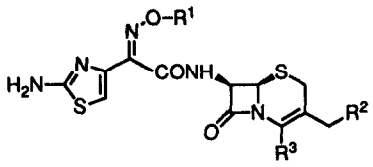
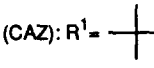
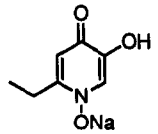
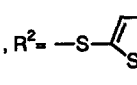


ceftazidime (CAZ): R^1=  , R^2=  , R^3= $-CO_2^-$

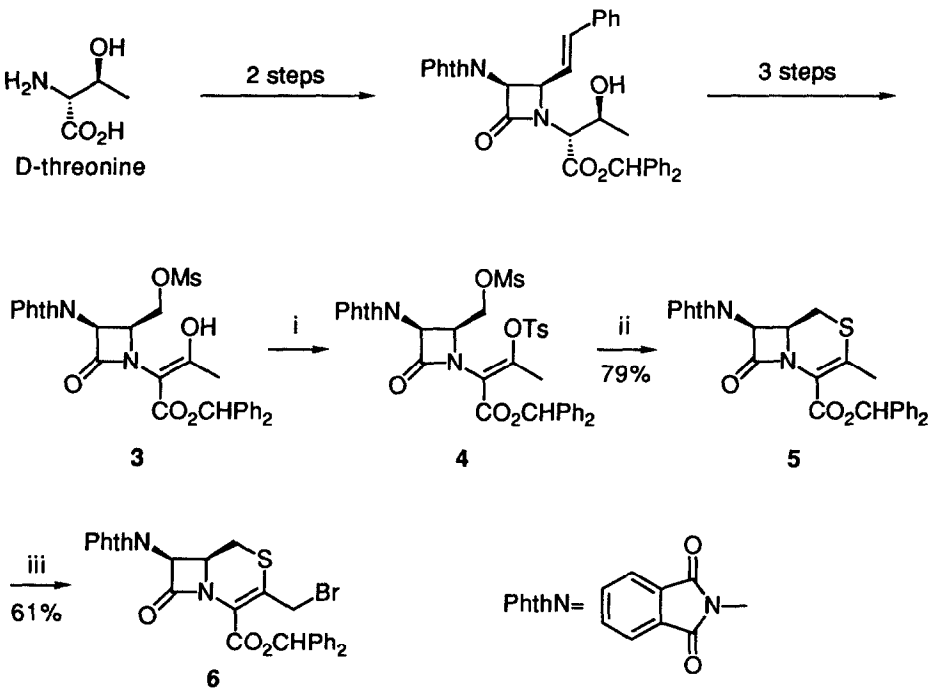
KP-736: R^1=  , R^2=  , R^3= $-CO_2Na$

Recently, such cephalosporins bearing catechol or related aromatics[3] at C-7 or C-3 as KP-736[4] have been reported concerning their potent activity against *P. aeruginosa* including resistant strains. With much interest in the anti-pseudomonal activity of the 2-isocephems having catechol or related aromatics, we synthesized optically active 2-isocephems with these substituents and examine their activity. In this communication, we describe the finding of excellent *in vitro* and *in vivo* antibacterial activity of 2-isocephems bearing a 1,3-dihydroxy-4-pyridone moiety at C-7 against *P. aeruginosa* including CAZ, AZT, or IPM resistant strains.

Synthesis

First, 3-bromomethyl derivative **6** which is the key intermediate in the synthesis of new optically active 2-isocephems was prepared. The lactam enol **3** derived in 5 steps from D-threonine (Scheme 1)[5] was tosylated with p-toluenesulfonyl chloride in the presence of *N*-methylpyrrolidine in CH_2Cl_2 at 0 ℃ to afford tosylate **4**. Treatment of **4** with hydrogen sulfide in CH_2Cl_2 at 5 ℃ provided (6*S*, 7*S*)-7-phthalimido-3-methyl-2-isocephem **5** in 79% yield from **3**. Free radical bromination of compound **5** by N-bromosuccinimide (NBS) in the presence

Scheme 1

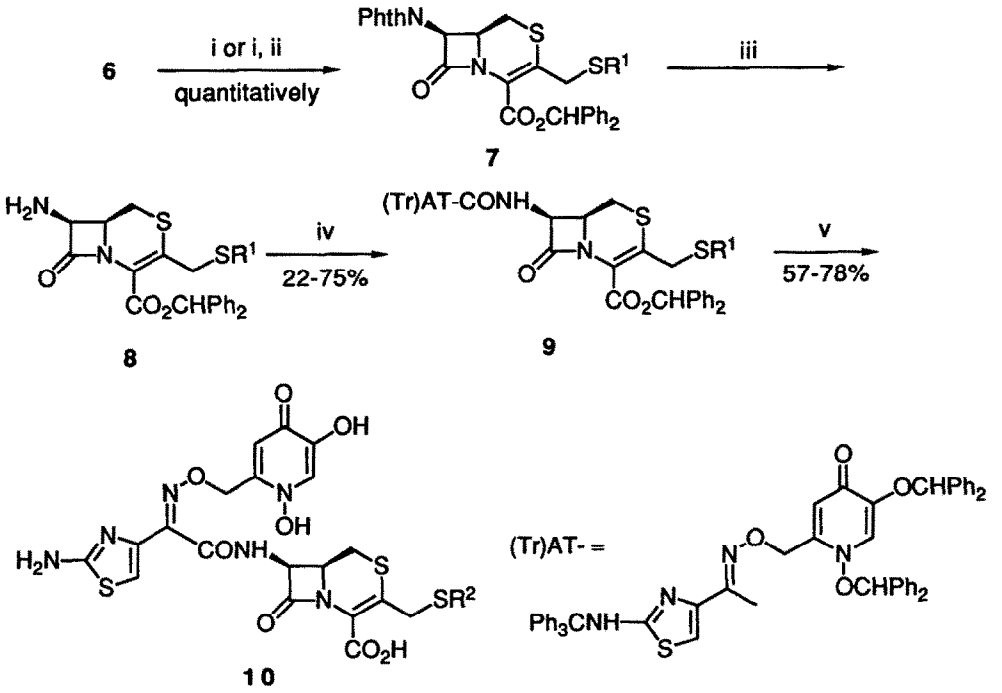


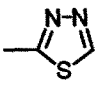
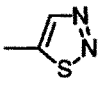
i: p-TsCl, N-methylpyrrolidine, 0℃ ; ii: H_2S, TEA, 5℃ ; iii: NBS, AIBN , $NaHCO_3$, reflux

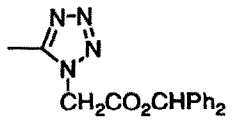
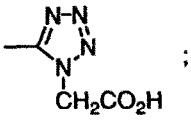
of 2,2'-azobis(isobutyronitrile) (AIBN) and $NaHCO_3$ in CCl_4 and $CHCl_3$ at reflux temperature gave 3-bromomethyl derivative **6** as stable crystalline solid in 61% yield.

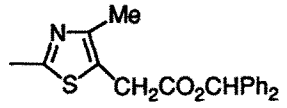
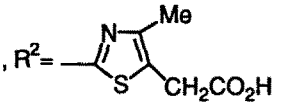
Next, we converted **6** into desired target compounds **10**. Thus, four 3-heterocyclic thiomethyl-2-isocephems **7** were prepared quantitatively by treatment of compound **6** with thiol derivative[6] in acetone-water at

Scheme 2



a: R^1=R^2=  ; b: R^1=R^2=  ;

c: R^1=  , R^2=  ;

d: R^1=  , R^2= 

i: HS-R^2, $NaHCO_3$, r.t. ; ii: Ph_2CN_2, r.t. ; iii: $MeNHNH_2$, -15℃ ; iv: (Tr)AT-CO_2H, DCC, HOBT, r.t. ; v: 1, TFA, anisole, r.t., 2, $NaHCO_3$

r.t.(Scheme 2). For the thiol derivatives having a carboxyl group, intermediary carboxylic acids were further esterified with diphenyldiazomethane to give **7c** and **7d**. Phthalimido group was removed by methylhydrazine in DMF at -15℃ to allow introduction of the 1,3-dihydroxy-4-pyridone moiety which brought about potent anti-pseudomonal activity. The resulting free amines **8** were coupled with active ester derived from protected 2-aminothiazole derivative[3a] bearing 1,3-dihydroxy-4-pyridone and 1-hydroxybenzotriazole (HOBT) in CH_2Cl_2 at r.t. to furnish compounds **9** in 22-75% yield from **7**. In the last step, triphenylmethyl and diphenylmethyl protective groups were removed at once with TFA in the presence of anisole at r.t. The resulting TFA salts were neutralized by $NaHCO_3$ to give desired 2-isocephems **10** in 57-78% yield.

Biological Assays

Compounds **10a-d** were evaluated for *in vitro* antibacterial activities against gram-negative (*Escherichia coli* NIHJ JC-2, *Klebsiella pneumoniae* NCTC-9632, *P. aeruginosa* ATCC-10145 and *P. aeruginosa* NCTC-10490) bacteria by using a two-fold agar dilution method.[7] In Table 1, their minimum inhibitory concentrations (MICs) are summarized and compared with those of CAZ.

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

Compd.	*E. coli* NIHJ JC-2	*K. pneumoniae* NCTC-9632	*P. aeruginosa* ATCC-10145	*P. aeruginosa* NCTC-10490
10a	0.2	≤0.025	0.1	≤0.025
10b	0.2	0.1	0.39	≤0.025
10c	0.05	≤0.025	≤0.025	≤0.025
10d	0.1	0.05	0.05	≤0.025
CAZ	0.39	0.05	1.56	1.56

As we would expect, 2-isocephems **10a-d** were found to possess potent antibacterial activity against gram-negative bacteria including *P. aeruginosa*. Especially, compounds **10c** and **10d** with a carboxyl group at C-3 side-chain were much more active against two strains of *P. aeruginosa* than CAZ and the range encompassed was from 32- to 64-fold. Furthermore, compound **10d** showed potent activity even against CAZ, AZT, or IPM resistant strains (Table 2).

Table 2. *In vitro* activity of **10d** against CAZ, AZT, or IPM resistant *P. aeruginosa* [MICs (μg/ml), Inoculum size: 10^6 cells/ml]

Organism	**10d**	CAZ	AZT	IPM
P. aeruginosa 59	1.56	50	25	50
P. aeruginosa 47	1.56	50	25	25
P. aeruginosa 56	1.56	12.5	50	25
P. aeruginosa 69	3.13	50	25	50

The protective effects of selected compounds **10d**, CAZ, and AZT were examined on systemic infection in mice caused by clinically isolated *P. aeruginosa* 58 and *P. aeruginosa* 67. One hour after intraperitoneal infection of bacteria with mucin, a single dose of each compound was administered to mice subcutaneously. The survival rates on day 7 were calculated and ED_{50} values were determined by the probit method. The results are showed in Table 3. Compound **10d** exhibited better *in vivo* efficacy on experimental infection caused by clinically isolated *P. aeruginosa* than CAZ or AZT by approximately 5-40-fold.

Table 3. Mouse Protection Test of **10d** in Comparison with CAZ and AZT

Test organism	Compounds	MIC (μg/ml)	Challenge dose (cells/mouse)	ED_{50} (mg/kg)
P. aeruginosa 58	**10d**	0.05	3.40×10^3	3.2
	CAZ	3.13	3.40×10^3	31.17
	AZT	12.5	3.40×10^3	122.35
P. aeruginosa 67	**10d**	0.1	6.30×10^5	21.8
	CAZ	1.56	6.30×10^5	>100
	AZT	1.56	6.30×10^5	>100

In summary, 2-isocephems bearing a 1,3-dihydroxy-4-pyridone moiety at C-7 possessed high *in vitro* potency against gram-negative organisms including *P. aeruginosa*. In addition, compound **10d** showed potent *in vitro* activity against CAZ, AZT, or IPM resistant *P. aeruginosa*, and better protective effects on systemic infection in mice caused by clinically isolated *P. aeruginosa* than reference compounds. Further investigation on the effect of 1,3-dihydroxy-4-pyridone and their analog connected 2-isocephems or 2-oxaisocephems on anti-pseudomonal activity is in progress.

Acknowledgment. The authors are grateful to Mr. S. Toyama for his kind and appropriate advice and to Mr. M. Matsumoto for biological data.

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(*Received in USA* 21 *April* 1994; *accepted* 19 *May* 1994)