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Synthesis and Biological Activity of New Optically Active 2-Oxaisocephems: 3-(*N*-Alkylpyridinium-4'-thio)methyl Derivatives

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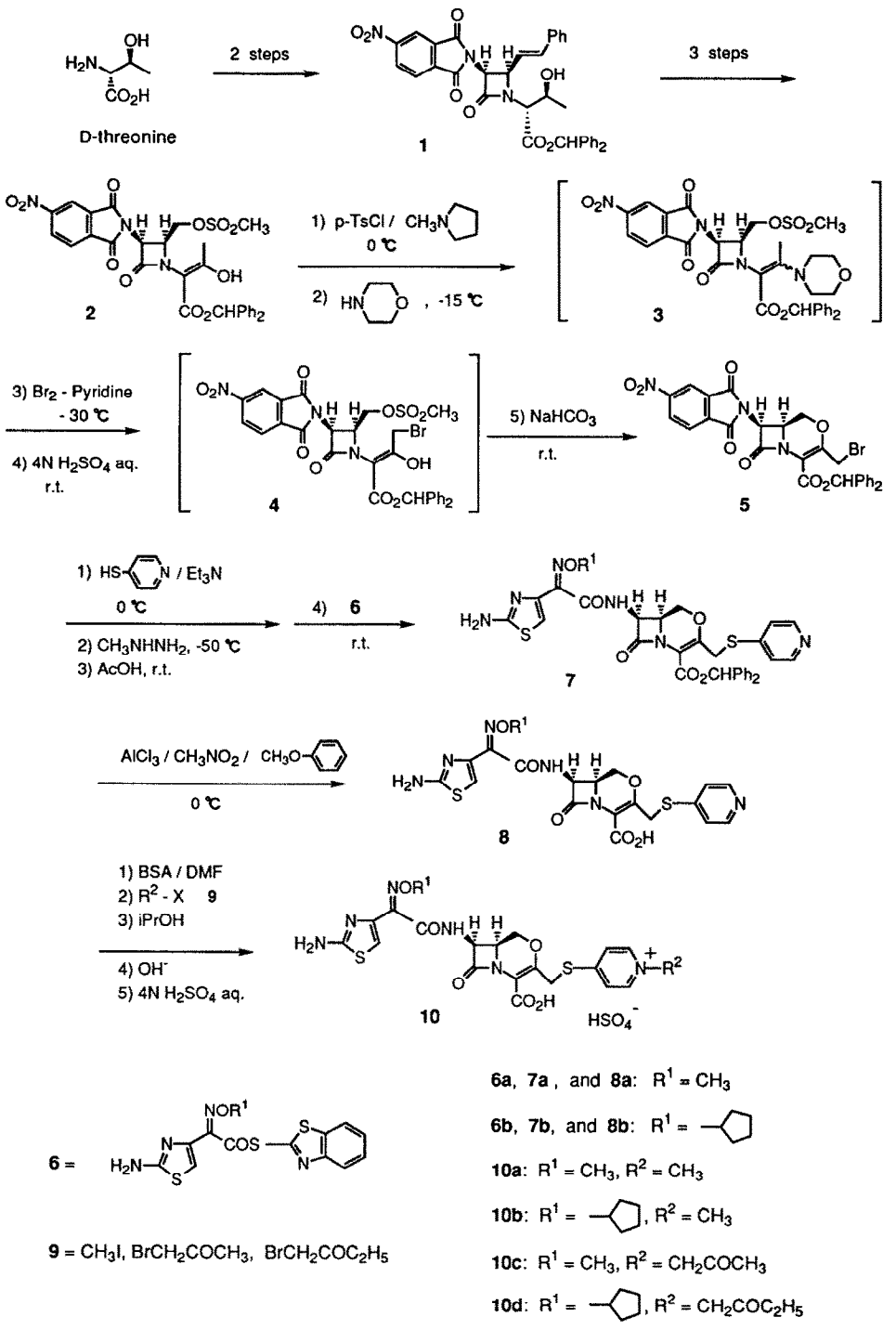
Abstract: A convenient synthesis of new optically active 2-oxaisocephems with (*N*-alkylpyridinium-4'-thio)methyl groups at the 3-position and their biological activities are described. In particular, **10b** and **10d** having [2-(2-aminothiazol-4-yl)-2-(Z)-cyclopentyloxyimino]acetamido group at the 7-position were found to possess high *in vitro* potency and showed excellent *in vivo* efficacy.

As part of our study to find more effective anti-infectives, we required a practical and convenient synthesis of optically active 2-oxaisocephem class of β-lactam antibiotics. A synthesis of optically pure 2-oxaisocephems with different substituents at the 3- and the 7-position is considered to be one of the most attractive subjects because of the expected enhancement of antibacterial activity.[1] Although 2-oxaisocephems have been reported to have only partial antibacterial activity,[2] most of previous reports were concerned on racemic compounds. And the existing enantioselective syntheses[3] are not appropriate since the introduction of various substituents into the 3-position is limited. Therefore, we intended to search more effective antibiotics which show broad spectrum of antibacterial activity by the synthesis of optically active 2-oxaisocephems with (*N*-alkylpyridinium-4'-thio)methyl groups at the 3-position and 2-aminothiazol-4-yl moiety at the 7-position. In particular, we attempted to improve antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) which is recently a major pathogen in hospitals and has been associated with an increasing number of infections since 1961. We describe herein the synthesis of new optically active 2-oxaisocephems and their biological activities.

Synthesis

Our synthetic strategy for the preparation of optically active 2-oxaisocephems involves the utilization of the key intermediate **5** having bromomethyl substituent at the 3-position and 4-nitrophthalimido group at the 7-position easily derived from the enol derivative **2** which was obtained in 5 steps from D-threonine *via* **1**.[4] Direct bromination of **2** as described in our preceding paper[4] was not applicable because benzhydryl ester of


D-threonine
2 steps
1
3 steps
2
1) p-TsCl / CH3N
0 ℃
2) HN O , -15 ℃
3
3) Br2 - Pyridine
- 30 ℃
4) 4N H2SO4 aq.
r.t.
4
5) NaHCO3
r.t.
5
1) HS N / Et3N
0 ℃
2) CH3NHNH2, -50 ℃
3) AcOH, r.t.
4) 6
r.t.
7
AlCl3 / CH3NO2 / CH3O
0 ℃
8
1) BSA / DMF
2) R2 - X 9
3) iPrOH
4) OH-
5) 4N H2SO4 aq.
10
HSO4-
6 =
9 = CH3I, BrCH2COCH3, BrCH2COC2H5
6a, 7a, and 8a: R1 = CH3
6b, 7b, and 8b: R1 =
10a: R1 = CH3, R2 = CH3
10b: R1 = , R2 = CH3
10c: R1 = CH3, R2 = CH2COCH3
10d: R1 = , R2 = CH2COC2H5

2 was unfortunately damaged. After various trials, we found that bromination of **3**[5] readily obtained from **2** gave a satisfactory result. The typical procedures to synthesize **5** are as follows: To a mixture of **2** (6.7 g, 10.5 mmol) and p-toluenesulfonyl chloride (2.21 g, 11.6 mmol) in CH_2Cl_2 (100 ml) was added *N*-methylpyrrolidine (990 mg, 11.6 mmol) at 0 ℃ dropwise. After stirring for 1 h, morpholine (3.66 g, 42 mmole) was added at -15 ℃ dropwise to the reaction mixture, which was stirred for 1.5 h to afford **3**. After workup, to a stirred solution of **3** in THF (100 ml) was added pyridine perbromide (2.51 g, 10.5 mmol) at -30 ℃. Then 4N aqueous sulfuric acid solution (70 ml) was added to the reaction mixture, which was stirred for 3 h at r.t. to give **4**. The thus obtained **4** was treated with $NaHCO_3$ (882 mg, 10.5 mmol) in acetone (70 ml) and H_2O (35 ml) at r.t. for 1 h to afford **5**[6] in 51% yield from **2**.

Next, we wished to convert **5** into desired target compounds **10** from the standpoint that the introduction of thio-substituted methyl groups into the 3-position and 2-aminothiazol-4-yl moiety into the 7-position was considered to contribute to the enhancement of antibacterial activity.[4] In order to introduce the 2-aminothiazol-4-yl moiety into the 7-position, it was required to deprotect 4-nitrophthalimido group. In our previous papers,[4,7] we reported that 4-nitrophthalimido group was smoothly removed by methylhydrazinolysis. After stirring of a mixture of **5** and an equimolar amount of 4-mercaptopyridine in the presence of triethylamine in DMF at 0 ℃ for 30 min, 1.1 equiv. of methylhydrazine was added at -50 ℃ dropwise. Then the reaction mixture was stirred for 30 min. The thus generated amine was allowed to react with equimolar amounts of 2-aminothiazole derivatives **6** in CH_2Cl_2 at r.t. to give **7**[8] in good yields (60-70% yields). To cleave the benzhydryl ester **7**, the use of aluminum trichloride[9] was proved to be efficient to obtain **8**.[10] Thus obtained **8** was easily converted into the target compounds **10**. After **8** was treated with 3 equiv. of *N,O*-(bistrimethylsilyl)acetamide (BSA) in DMF at r.t. for 1 h, halides **9** were added to the mixture, which was stirred at 0 ℃ - r.t. for 6 h to give **10**[11] (55-65% yields). Compounds **10** were isolated as hydrogensulfates.

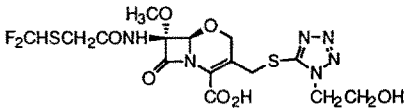
In summary, we established the convenient synthetic method of new optically active 2-oxaisocephems from **2** *via* the key intermediate **5** and this method was applicable to obtain various compounds with different (*N*-alkylpyridinium-4'-thio)methyl groups at the 3-position.

Biological Assays

Compounds **10a-d** were tested for *in vitro* antibacterial activities against gram-positive (*Staphylococcus aureus* FDA 209P and MRSA 57) and gram-negative (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* ATCC 10145) bacteria. Their minimum inhibitory concentrations (MICs: μg/ml, inoculum size: 10^6 cells/ml) were determined by twofold agar dilution method.[12] The results are summarized in Table 1. The antibacterial activitiy of flomoxef and vancomycin as reference compounds is also presented. 2-Oxaisocephems **10a-d** have broader spectrum of antibacterial activities than the corresponding 1-oxacephems, flomoxef. Especially, **10b** and **10d** with [2-(2-aminothiazol-4-yl)-2-(Z)-cyclopentyloxyimino]acetamido group at the 7-position showed well-balanced and potent activity against test organisms including MRSA. Vancomycin is not effective against gram-negative bacteria. And substitution of the 7-position by [2-(2-aminothiazol-4-yl)-2-(Z)-

Table 1. *In Vitro* Antibacterial Activity (MICs, μg/ml)

Compd.	*S. aureus* FDA 209P	MRSA 57	*E. coli* NIHJ JC-2	*P. aeruginosa* ATCC 10145
10a	0.2	50	< 0.025	6.25
10b	0.05	3.13	0.39	3.13
10c	0.39	100	< 0.025	12.5
10d	0.2	6.25	0.39	6.25
flomoxef	0.2	25	0.1	> 100
vancomycin	0.78	1.56	>100	>100



flomoxef

methoxyimino]acetamido group (**10a** and **10c**) increased the activity against *E. coli*, but it caused a decrease in the activity against MRSA.

Efficacy of the selected compounds **10b**, **10d**, flomoxef, and vancomycin in systemic infection caused by *S. aureus* Smith, *E. coli* No.29, and clinical isolated MRSA 5038 (low-resistant), MRSA 5120 (high-resistant), and MRSA 5129 (high-resistant) in mice is shown in Table 2. One hour after intraperitoneal infection, several doses of each compound were subcutaneously administered to mice (a group of 10 animals). Efficacy of each compound was expressed as fifty percent effective dose values (ED_{50}) which were calculated from the survivals

Table 2. Mouse Protection Test of **10b** and **10d** in Comparison with flomoxef and vancomycin

test organism	Compounds	MIC (μg/ml)	Challenge dose (cells/mouse)	ED_{50} (mg/kg)	95% confidence limits (mg/kg)
S. aureus Smith	**10b**	0 39	1.32×10^7	0.08	0.04-0 12
	10d	0.2		0.22	0.11-0.37
	flomoxef	0.39		0.53	0.17-0.87
	vancomycin	0.78		3.74	2.58-5.12
E. coli No.29	**10b**	0.39	1.35×10^6	0.09	0.05-0.12
	10d	0.39		0.15	0.12-0.19
	flomoxef	0.1		0.43	0.31-0.57
MRSA 5038	**10b**	3 13	1.0×10^7	3.40	0.29-4.73
	vancomycin	1.56		2.70	1.90-4.00
MRSA 5120	**10d**	6.25	7.6×10^7	1.00	0.60-1.57
	vancomycin	0.78		11.49	7.12-17.25
MRSA 5129	**10b**	6.25	1.31×10^8	3.91	0.92-9.15
	vancomycin	0.78		18.68	9.38-27.38

on the seventh day after infection by the probit method. The *in vivo* efficacy of the compound **10b** and **10d** on the experimental infection caused by *S. aureus* Smith and *E. coli* No.29 was greater than that of flomoxef or vancomycin. Vancomycin is widely used clinically as an anti-MRSA agent. Although vancomycin has higher MIC values than **10b** and **10d** against MRSA, *in vivo* potency of **10b** and **10d** on systemic infection caused by high-resistant MRSA was superior to that of vancomycin. The outstanding *in vivo* efficacy of **10b** and **10d** would be due to a bactericidal activity against MRSA, while vancomycin shows a bacteriostatic activity. This evidence will be reported in detail elsewhere.

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6. **5**: white needles, mp = 187-188.5 ℃ (decomp.). $[\alpha]_D^{27}$ = - 35.4 (c = 0.226, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 3.94-4.08 (1H, m), 4.30-4.50 (2H, m), 4.55 (1H, dd, J = 4, 10.3 Hz), 4.72 (1H, d, J = 10.5 Hz), 5.97 (1H, d, J = 5.4 Hz), 6.97 (1H, s), 7.20-7.60 (10H, m), 8.11 (1H, d, J = 8.1 Hz), 8.67 (1H, dd, J = 2, 8.1 Hz), 8.71 (1H, d, J = 2 Hz). Anal. Calcd. for $C_{29}H_{20}BrN_3O_8$: C, 56.33, H, 3.26, N, 6.79. Found: C, 56.25, H, 3.15, N, 6.80.
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8. **7a**: ^1H NMR (250 MHz, $CDCl_3$): δ 3.89–4.20 (5H, m), 4.23 (1H, d, J = 14 Hz), 4.35 (1H, d, J = 14 Hz), 4.62 (1H, dd, J = 4.9, 11.1 Hz), 5.86 (1H, dd, J = 4.9, 7.6 Hz), 6.68 (1H, s), 6.93 (1H, s), 7.11 (2H, dd, J = 1.6, 4.7 Hz), 7.25-7.55 (10H, m), 8.27 (2H, dd, J = 1.6, 4.7 Hz), 8.64 (1H, d, J = 7.6 Hz).
7b: ^1H NMR (250 MHz, $CDCl_3$): δ 1.42-1.87 (8H, m), 3.96-4.11 (2H, m), 4.30 (2H, s), 4.64 (1H, dd, J = 2.8, 10 Hz), 4.75-4.85 (1H, m), 5.67 (1H, dd, J = 4.7, 6.3 Hz), 6.74 (1H, s), 6.94 (1H, s), 7.14 (2H,

dd, J = 1.6, 4.7 Hz), 7.26-7.55 (10H, m), 8.06 (1H, d, J = 6.3 Hz), 8.29 (2H, dd, J = 1.6, 4.7 Hz).

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10. **8a:** ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.87–4.20 (5H, m), 4.25 (1H, d, J = 14 Hz), 4.40 (1H, d, J = 14 Hz), 4.47 (1H, dd, J = 2.9, 10.2 Hz), 5.85 (1H, dd, J = 4.6, 8.2 Hz), 6.69 (1H, s), 7.32 (2H, d, J = 6.2 Hz), 8.36 (2H, d, J = 6.2 Hz), 9.15 (1H, d, J = 8.2 Hz).
8b: ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.40–1.88 (8H, m), 3.85–4.10 (2H, m), 4.27 (1H, d, J = 14.1 Hz), 4.42 (1H, d, J = 14.1 Hz), 4.49 (1H, dd, J = 2.8, 10 Hz), 4.60-4.75 (1H, m), 5.63 (1H, dd, J = 4.5, 8.3 Hz), 6.74 (1H, s), 7.38 (2H, d, J = 6.2 Hz), 8.38 (2H, d, J = 6.2 Hz), 9.13 (1H, d, J = 8.3 Hz).
11. **10b:** ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.40-1.85 (8H, m), 3.87-4.05 (2H, m), 4.19 (3H, s), 4.47-4.73 (4H, m), 5.65 (1H, dd, J = 4.4, 8.2 Hz), 6.79 (1H, s), 8.03 (2H, d, J = 7 Hz), 8.70 (2H, d, J = 7 Hz), 9.19 (1H, d, J = 8.2 Hz).
10d: ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.03 (3H, t, J = 7.2 Hz), 1.40-1.87 (8H, m), 2.64 (2H, q, J = 7.2 Hz), 3.90-4.14 (2H, m), 4.35-4.80 (4H, m), 5.57 (2H, s), 5.66 (1H, dd, J = 4.3, 8.1 Hz), 6.79 (1H, s), 8.11 (2H, d, J = 7.2 Hz), 8.52 (2H, d, J = 7.2 Hz), 9.19 (1H, d, J = 8.2 Hz).
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