SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY OF 7α-HYDROXYETHYL CEPHEM SULFONE AND SULFOXIDE DERIVATIVES

SHINTARO NISHIMURA, NOBUYOSHI YASUDA, HIROSHI SASAKI, YOSHIMI MATSUMOTO, TOSHIAKI KAMIMURA, KAZUO SAKANE and TAKAO TAKAYA

New Drug Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

(Received for publication September 12, 1989)

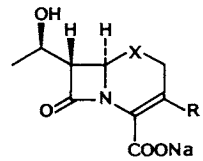
Our recent work[1,2] demonstrated the synthesis and biological activity of 7α-hydroxyethyl cephem (**1**) and 1-oxacephem (**2**). Cephem (**1**) and 1-oxacephem (**2**) were found to have potent β-lactamase inhibitory activity. Therefore, we became interested in studying the properties of other analogues of **1** and **2**, because sulbactam (**3**)[3], which is widely used in the clinic as a β-lactamase inhibitor, has a sulfone structure at the 1 position. Thus, the biological and structural characteristics of **1**, **2** and **3** prompted us to study the properties of 7α-hydroxyethyl cephem sulfone and sulfoxide having a formyl group as an electron-withdrawing group (EWG) at the 3 position.

The main focus of this report was to investigate the relationship between β-lactamase inhibitory activity and oxidative state at the 1 position of 7α-hydroxyethyl cephem derivatives.

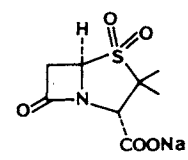
Preparation of the cephem derivatives is illustrated in Scheme 1. The starting material, compound **4** was prepared by our method[1]. Compound **4** was deprotected to give sodium salt **5** in 37% yield by the palladium(0)-catalyzed exchange deprotection method[4]. Sulfone **9** was obtained by treatment of **4** with 2.2 equivalent of *m*-chloroperbenzoic acid (*m*-CPBA) in 72% yield. However, subsequent deprotection of **9** under the same condition described above resulted in failure to give degradation products.

Treatment of **4** with 1.1 equivalent of *m*-CPBA gave a 1*R*, 1*S* mixture of sulfoxides, **6** and **7**. The mixture was chromatographed on silica gel to give **6** (less polar, 24% yield) and **7** (more polar, 36% yield). Based upon the previous ^{1}H NMR studies of cephalosporin *R*- and *S*-sulfoxides[5], the less polar **6** was assigned to *R* stereochemistry (^{1}H NMR ($CDCl_3$) δ 3.29 (1H, d, J=17 Hz, 2-H$_\alpha$), 4.64 (1H, d, J=3 Hz, 6-H), 4.72 (1H, d, J=17 Hz, 2-H$_\beta$)) and the more polar **7** was assigned to *S* stereochemistry (^{1}H NMR ($CDCl_3$) δ 2.95 (1H, dd, J=2 and 17 Hz, 2-H$_\alpha$), 4.54 (1H, dd, J=2 and 3 Hz, 6-H), 4.47 (1H, d, J=17 Hz, 2-H$_\beta$)). The *S*-sulfoxide **7** could be deprotected to give **8** in 47% yield. However, deprotection of the *R*-sulfoxide **6** resulted in failure similarly to that of sulfone **9**.

Fig. 1. Structures of **1**, **2** and **3**.



1 X=S R=EWG
2 X=O R=EWG



3

All compounds were found to possess β-lactamase inhibitory activity but no antibacterial activity.

Table 1 shows the β-lactamase inhibitory activity of the synthetic compounds, sulbactam and clavulanic acid. Allyl ester **4** and **7** have more potent β-lactamase inhibitory activity against both of TEM penicillinase (PCase) and cephalosporinases (CSase) than the corresponding sodium salts **5** and **8**.

Among these compounds, the sulfide **4** has the most potent β-lactamase inhibitory activity against CSase and the *R*-sulfoxide **6** has the most potent inhibitory activity against TEM PCase. Interestingly, the *R*-sulfoxide **6** has potent β-lactamase inhibitory activity in comparison with the *S* isomer **7**.

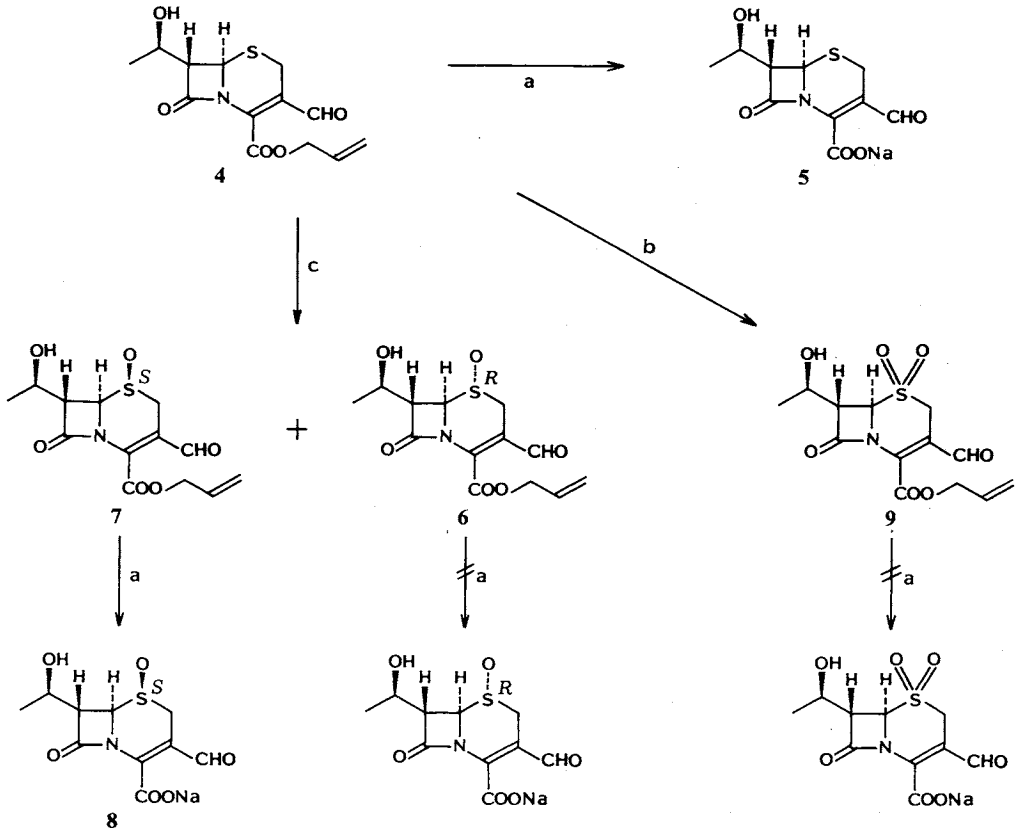
The sulfone **9** brings about 5-fold increase of TEM PCase inhibitory activity and 10-fold decrease of Ic CSase inhibitory activity based upon sulfide **4**. These tendencies are similar to that of *N*-alkylaminopenicillanic acid derivatives, which were reported by the Leo group[6].

In summary, we found that β-lactamase inhibitory activity of 7α-hydroxyethyl cephem derivatives is influenced by the oxidative state at the 1 position and its stereochemical configuration, that is, the *R*-sulfoxide **6** has the most potent TEM PCase inhibitory activity, and the sulfides **4**, **5** and sulfone **9** have more potent CSase inhibitory activity than the *R*-sulfoxide **6**. The *S*-sulfoxides **7** and **8** have little β-lactamase inhibitory activity.

Experimental

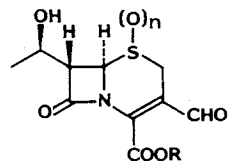
MP's were measured with a Yanagimoto micro melting point apparatus and are uncorrected. IR

Scheme 1.



(a) PPh_3, sodium 2-ethylhexanoate, $Pd(PPh_3)_4$, (b) 2.2 equiv of *m*-CPBA, (c) 1.1 equiv of *m*-CPBA.

Table 1. β-Lactamase inhibitory activity[a] of 7α-hydroxyethyl cephem derivatives.



Compound No.	n	R	ID_{50} (μg/ml)[b]			
			TEM PCase	Ia CSase	Ib CSase	Ic CSase
4	0	$CH_2CH{=}CH_2$	63	<0.125	0.62	0.43
5	0	Na	>125	<0.5	1.6	0.65
6	1(1*R*)	$CH_2CH{=}CH_2$	4.0	2.4	4.5	<0.5
7	1(1*S*)	$CH_2CH{=}CH_2$	140	>500	>500	21
8	1(1*S*)	Na	>500	>500	>500	90
9	2	$CH_2CH{=}CH_2$	12	<0.5	0.56	4.3
Sulbactam			1.2	42	14	<0.5
Clavulanic acid			1.0	12	7.8	0.6

[a] Serial dilution of a β-lactamase inhibitor were incubated with enzyme solution for 10 minutes at 37°C. Residual β-lactamase activity was determined spectrophotometrically using the chromogenic substrate nitrocefin (50 μg/ml) at 482 nm. ID_{50} was calculated as the concentration inhibiting 50% of activity.

[b] TEM PCase: *Escherichia coli* 18, Ia CSase: *Enterobacter cloacae* 91, Ib CSase: *E. coli* HB101/pCF3, Ic CSase: *Proteus vulgaris* 9.

spectra were recorded with a Hitachi 260-10 spectrometer. ^{1}H NMR were recorded using a Hitachi R-90H spectrometer. Chemical shifts (δ) are reported in ppm from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS, in D_2O) or TMS (in $CDCl_3$) as internal standard.

Sodium 7α-[(1*R*)-1-Hydroxyethyl]-3-formyl-3-cephem-4-carboxylate (**5**)

To a solution of allyl 7α-[(1*R*)-1-hydroxyethyl]-3-formyl-3-cephem-4-carboxylate (**4**)[1] (200 mg), triphenylphosphine (17.6 mg), sodium 2-ethylhexanoate (135 mg) in dry ethyl acetate (1.4 ml) was added tetrakis(triphenylphosphine)palladium(0) (15.8 mg) at 0°C under an atmosphere of nitrogen. The mixture was stirred for 40 minutes at room temperature and poured into water (20 ml). The aqueous layer was separated, washed with ethyl acetate and lyophilized. The powder was purified by column chromatography (non-ionic adsorption resin; Diaion HP-20 (40 ml), eluent; water). Freeze-drying of the product fractions gave 69.2 mg (37%) of **5**: MP 125~130°C (dec): IR (Nujol) cm^{-1} 3350, 1770, 1650, 1620, 1585, 1340; ^{1}H NMR (D_2O) δ 1.28 (3H, d, J=7 Hz), 3.35 (1H, d, J=17 Hz), 3.63 (1H, dd, J=3 and 5 Hz), 3.81 (1H, d, J=17 Hz), 4.34 (1H, q, J=7 Hz), 4.94 (1H, d, J=3 Hz), 9.45 (1H, s).

Allyl 7α-[(1*R*)-1-Hydroxyethyl]-3-formyl-3-cephem-4-carboxylate 1,1-Dioxide (**9**)

To a solution of **4** (150 mg, 0.5 mmol) in dry ethyl acetate (4.5 ml) was added *m*-CPBA (240 mg) at 0°C. The mixture was stirred at room temperature for 3 hours. Excess amount of dimethylsulfide was added to the mixture. After stirring for a few minutes, the mixture was diluted with ethyl acetate (40 ml), washed with aqueous sodium hydrogen carbonate and sodium chloride, dried over magnesium sulfate and evaporated. The residue was purified by silica gel column chromatography (Wakogel C-200; 25 g, eluent; dichloromethane) to give yellow oil of **9** (119 mg, 72% yield): IR (CH_2Cl_2) cm^{-1} 3600, 1810, 1740, 1670, 1610, 1370, 1340, 1295, 1230, 1205; ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, d, J=7 Hz), 1.50~2.30 (1 H, br s), 3.76 (1H, dd, J=2 and 18 Hz), 3.98 (1 H, dd, J=3 and 4 Hz), 4.30~4.57 (1H, m), 4.72~4.94 (2H, m), 5.00 (1H, m), 5.20~5.52 (1H, m), 5.37 (1H, d, J=18 Hz), 5.72~6.20 (1H, m), 9.75 (1H, s).

Allyl 7α-[(1*R*)-1-Hydroxyethyl]-3-formyl-3-cephem-4-carboxylate 1*R*- (**6**) and 1*S*-Oxide (**7**)

To a solution of **4** (300 mg, 1.0 mmol) in dry ethyl acetate (9 ml) was added *m*-CPBA (240 mg) at −20°C. The mixture was stirred at −20°C for 30 minutes. Excess amount of dimethylsulfide was added to the mixture. After stirring for a few minutes, the mixture was diluted with ethyl acetate (80 ml), washed with aqueous sodium hydrogen carbonate and sodium chloride, dried over magnesium sulfate and evaporated. The residue was purified by silica gel column chromatography (Wakogel C-200; 15 g, eluent; dichloromethane). The *R*-sulfoxide **6** was eluted first and obtained as oil (76 mg, 24% yield): IR (CH_2Cl_2) cm^{-1} 3620, 1800, 1740, 1680, 1610, 1410, 1380, 1060; ^{1}H NMR ($CDCl_3$) δ 1.42 (3H, d, J=7 Hz), 1.63 (1H, br s), 3.29 (1H, d, J=17 Hz, 2-H_α), 3.81 (1H, dd, J=3 and 4 Hz, 7-H), 4.30~4.55 (1H, m), 4.64 (1H, d, J=3 Hz, 6-H), 4.72 (1H, d, J=17 Hz, 2-H_β), 4.75~4.88 (2H, m), 5.20~5.56 (2H, m), 5.73~6.20 (1H, m), 9.65 (1H, s).

The more polar *S*-sulfoxide **7** was obtained as amorphous powder (114 mg, 36%): IR (CH_2Cl_2) cm^{-1} 3620, 1800, 1740, 1680, 1610, 1400, 1380, 1060; ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, d, J=7 Hz), 2.30 (1H, br s), 2.95 (1H, dd, J=2 and 17 Hz, 2-H_α), 3.80 (1H, dd, J=3 and 5 Hz, 7-H), 4.23~4.48 (1H, m), 4.54 (1H, dd, J=2 and 3 Hz, 6-H), 4.47 (1H, d, J=17 Hz, 2-H_β), 4.78~4.94 (2H, m), 5.20~5.50 (2H, m), 5.75~6.20 (1H, m), 9.85 (1H, s).

Sodium 7α-[(1*R*)-1-Hydroxyethyl]-3-formyl-3-cephem-4-carboxylate 1*S*-Oxide (**8**)

Compound **8** was prepared from the *S*-sulfoxide **7** in 47% yield as described for **5**.

MP 110~120°C (dec): IR (Nujol) cm^{-1} 1780, 1660, 1620, 1460, 1380, 1330, 1040; ^{1}H NMR (D_2O) δ 1.33 (3H, d, J=7 Hz), 3.28 (1H, dd, J=2 and 18 Hz, 2-H_α), 3.87 (1H, dd, J=3 and 5 Hz, 7-H), 4.32 (1H, m), 4.37 (1H, d, J=18 Hz, 2-H_β), 4.87 (1H, m), 8.62 (1H, s).

References

1) NISHIMURA, S.; N. YASUDA, H. SASAKI, Y. MATSUMOTO, T. KAMIMURA, K. SAKANE & T. TAKAYA: Synthesis and β-lactamase inhibitory activity of 7α-hydroxyethyl cephem derivatives. J. Antibiotics 42: 159~162, 1989

2) NISHIMURA, S.; H. SASAKI, N. YASUDA, Y. MATSUMOTO, T. KAMIMURA, K. SAKANE & T. TAKAYA: Synthesis and biological activity of 7α-hydroxyethyl-1-oxacephem derivatives. J. Antibiotics 42: 1124~1132, 1989

3) ENGLISH, A. R.; J. A. RETSEMA, A. E. GIRARD, J. E. LYNCH & W. E. BARTH: CP-45,899, a beta-lactamase

inhibitor that extends the antibacterial spectrum of beta-lactams: Initial bacteriological characterization. Antimicrob. Agents Chemother. 14: 414～419, 1978

4) JEFFEREY, P. D. & S. W. MCCOMBIE: Homogeneous, palladium(0)-catalyzed exchange deprotection of allylic esters, carbonates, and carbamates. J. Org. Chem. 47: 587～590, 1982

5) DEMARCO, P. V. & R. NAGARAJAN: Chapter 8. Physical-chemical properties of cephalosporins and penicillins. *In* Cephalosporins and Penicillins. Chemistry and Biology. *Eds.*, E. H. FLYNN, pp. 312～369, Academic Press, 1972

6) CALVERLEY, M. J. & M. BEGTRUP: *N*-(Functionalized alkyl) derivatives of 6-aminopenicillanic acid: A new series of specific inhibitors of β-lactamase from *Enterobacter cloacae* P99. J. Antibiotics 36: 1507～1515, 1983