KY-109, A NEW BIFUNCTIONAL PRO-DRUG OF A CEPHALOSPORIN

CHEMISTRY, PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES†

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(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-*O*-(L-alanyl)mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate hydrochloride (KY-109) was synthesized as a bifunctional pro-drug designed to improve the oral absorption of the parent drug (KY-087), a cephalosporin similar in activity to cefamandole. The pro-drug was found to possess the desired factors for an orally active pro-drug, that is, appropriate solubility, lipophilicity and ease hydrolysis into the parent drug. As predicted from these factors, the pro-drug when administered orally to rats was well absorbed, and gave high blood levels of the parent drug.

7-(D-Mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylic acid (KY-087)[1] is a cephalosporin with a broad spectrum of antibacterial activity *in vitro*. It is, however, poorly absorbed from the gastro-intestinal tract after oral administration, probably due to its low lipophilicity.

An ester pro-drug approach is frequently utilized as a method for increasing the lipophilicity of a drug and thereby improving its absorption after oral administration. A β-lactam antibiotic containing a relatively basic amino group can give an ester with high lipophilicity and sufficient aqueous solubility, which is significantly better absorbed orally than the parent drug[1]. Examples of such successful compounds are the pivaloyloxymethyl[3], 1-ethoxycarbonyloxyethyl[4], phtalidyl[5] and (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl[6] esters of ampicillin. In contrast, esters of β-lactam antibiotics lacking a basic amino group in the side chain, such as KY-087, generally seem to be weakly active when given orally, probably because they lack sufficient solubility in water for efficient absorption from the gastro-intestinal tract to occur[2,7]. To overcome the low aqueous solubility of such esters of KY-087, we have prepared the bifunctional pro-drug, (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-*O*-(L-alanyl)mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate hydrochloride, having in addition to the ester group in the 4-position an L-alanine ester formed with the side chain α-hydroxyl group. Due to the amino group in the alanine residue, the bifunctional pro-drug might be expected to have increased aqueous solubility along with a lipophilic character.

In this report we describe the physico-chemical and biological properties of the bifunctional pro-drug KY-109 in comparison with those of the parent drug (KY-087) and the monofunctional pro-drug (KY-106) having only the ester group in the 4-position.

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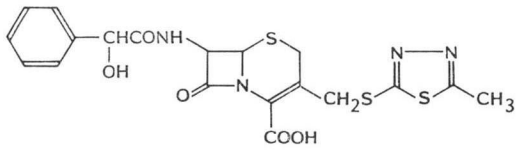
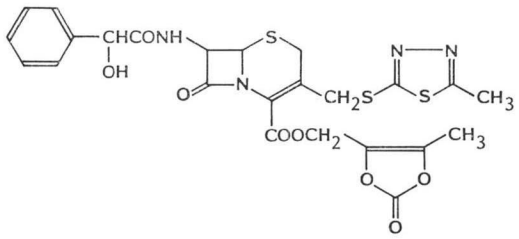
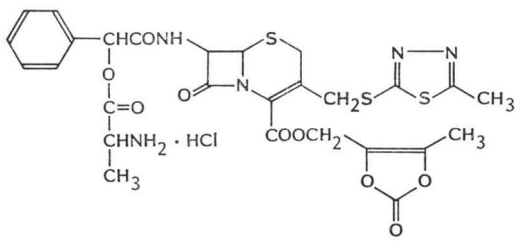
Chart 1.



Parent drug, KY-087 (**1**)



Monofunctional pro-drug, KY-106 (**2**)



Bifunctional pro-drug, KY-109 (**3**)

Chemistry

Three general pathways were followed in the synthesis of KY-109 (Chart 2). In one of these (method A) the starting material was KY-087, which was treated with 4-bromomethyl-5-methyl-1,3-dioxol-2-one in the presence of potassium acetate in dimethylformamide (DMF) to form KY-106. Treatment of KY-106 with *N*-(*tert*-butoxycarbonyl)-L-alanine in the presence of dicyclohexylcarbodiimide (DCC) in dichloromethane yields the key intermediate (**8**) having a protected amino group. The intermediate **8** was treated with HCl-acetone to remove the protecting group to give KY-109, which was isolated as the hydrochloride.

In the second pathway (method B) potassium 7-amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl]-3-cephem-4-carboxylate(7-ACTD K) was treated with ethyl acetoacetate followed by 4-bromomethyl-5-methyl-1,3-dioxol-2-one to give **4**, which was converted to **5** by treatment with HCl in methanol-dichloromethane. Treatment of the latter with *O*-[*N*-(*tert*-butoxycarbonyl)-L-alanyl]-D-mandelic acid (**6**) in the presence of DCC in dichloromethane gave the intermediate **8**, which was converted to KY-109 as in method A.

In the third pathway (method C) compound **7** was prepared by reaction of 7-amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylic acid (7-ACTD) with a mixed anhydride of **6** in the presence of triethylamine in acetone. The compound **7** was converted to the intermediate **8** by treatment with 4-bromomethyl-5-methyl-1,3-dioxol-2-one in the presence of potassium acetate in DMF.

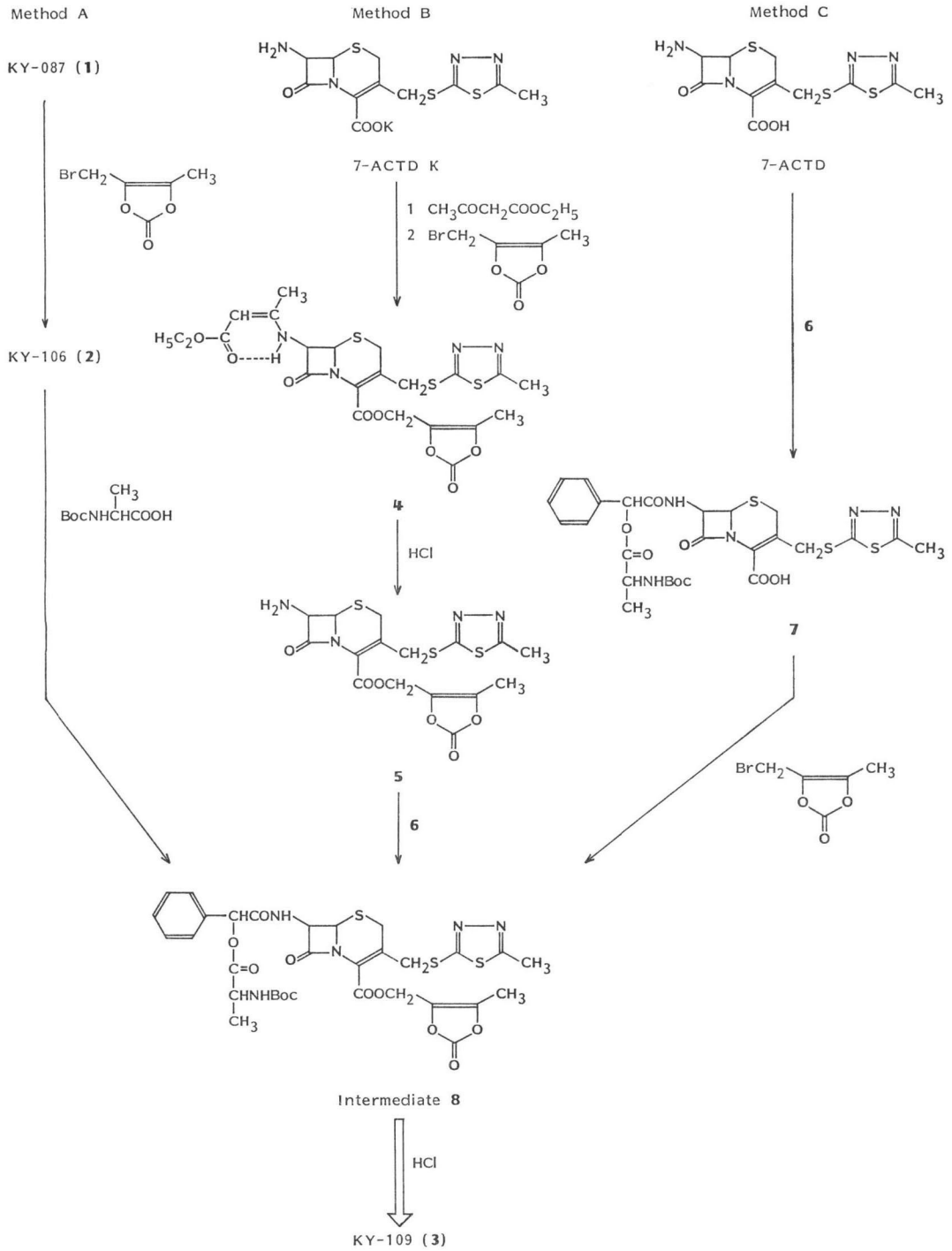
Results

In Vitro Antibacterial Activity

The *in vitro* antibacterial activity of the parent drug KY-087 was compared with that of the widely used oral cephalosporins, *i.e.*, cefaclor (CCL) and cephalexin (CEX), and of the parenteral cephalosporin, cefamandole (CMD) (Table 1).

The activity of KY-087 against the Gram-positive bacteria, *Staphylococcus aureus, Streptococcus pneumoniae, Micrococcus luteus* and *Bacillus subtilis*, was superior to that of CCL and CEX, and similar to that of CMD. Against the Gram-negative bacteria *Escherichia coli, Proteus mirabilis* and *P. vulgaris*, KY-087 was superior to CCL and CEX, and marginally less active than CMD. Against *Haemophilus influenzae*, KY-087 was highly effective; its activity was equal to that of cefamandole and 4~32 times that of the oral cephalosporins tested.

Chart 2.



Aqueous Solubility, Lipophilicity and Hydrolysis

The aqueous solubility and lipophilicity of KY-087, the monofunctional pro-drug KY-106 and the bifunctional pro-drug KY-109 are shown in Table 2. KY-087 sodium and KY-109 were soluble in water

Table 1. Antibacterial activity of KY-087 against standard strains of bacteria.

Organism	MIC (μg/ml)*			
	KY-087	Cefamandole	Cefaclor	Cephalexin
Staphylococcus aureus 209-P	0.10	0.20	1.56	3.13
S. aureus Smith	0.20	0.39	1.56	3.13
S. aureus No. 80	0.39	0.78	3.13	3.13
Streptococcus pneumoniae III	0.78	0.78	3.13	6.25
Micrococcus luteus ATCC 9341	0.05	0.02	0.01	0.05
Bacillus subtilis ATCC 6633	0.02	0.02	0.10	0.39
Escherichia coli NIHJ JC-2	1.56	0.78	1.56	6.25
E. coli NIH	0.20	0.10	0.78	3.13
E. coli K-12	0.10	0.10	0.78	3.13
E. coli KC-14	0.39	0.20	0.78	3.13
E. coli No. 8	0.78	0.39	1.56	6.25
E. coli No. 24	0.78	0.39	1.56	3.13
Klebsiella pneumoniae KC-1	0.39	0.20	0.39	3.13
K. pneumoniae NCTC 9632	0.39	0.20	0.39	3.13
Serratia marcescens IFO 3736	100	25	>100	100
Proteus vulgaris OX-19	0.78	0.39	6.25	12.5
P. mirabilis 1287	0.20	0.20	0.78	6.25
P. morganii Kono	25	12.5	>100	>100
Haemophilus influenzae N-17	0.39	0.39	1.56	6.25
H. influenzae S-31	0.78	0.78	3.13	25

* Determined with an inoculum of 10^6 cfu/ml.

Table 2. Physico-chemical properties and hydrolysis data of KY-087, KY-106 and KY-109.

Drug	Aqueous solubility (mg/ml)	Lipophilicity, 1-octanol - H_2O (pH 6.5)	Hydrolysis, $t_{1/2}$ (minutes)	
			In 1% rat serum	In 30% rat intestinal homogenate
KY-087 (Parent drug)	>500	0.0098	—	—
KY-106 (Monofunctional pro-drug)	0.02	39.0	NT	NT
KY-109 (Bifunctional pro-drug)	>500	7.8	3.7	2.2

NT: Not tested.

to the extent of 500 mg/ml or greater, whereas KY-106 had a very low solubility. The lipophilicity, expressed as the partition coefficient between 1-octanol and pH 6.5 phosphate buffer, was 39.0 for KY-106 and 7.8 for KY-109, compared with less than 0.01 for KY-087.

KY-109 was hydrolyzed to the parent drug in serum and intestinal homogenate of the rat, with half-lives of 3.7 and 2.2 minutes, respectively (Table 2).

The stability of KY-109 at pH values between 2 and 7, measured at 35°C by determining residual intact pro-drug, is shown in Fig. 1. The compound was stable in acidic solution but was rather rapidly destroyed in neutral solution, with a half-life of 1.1 hours.

Oral Absorption in Rats

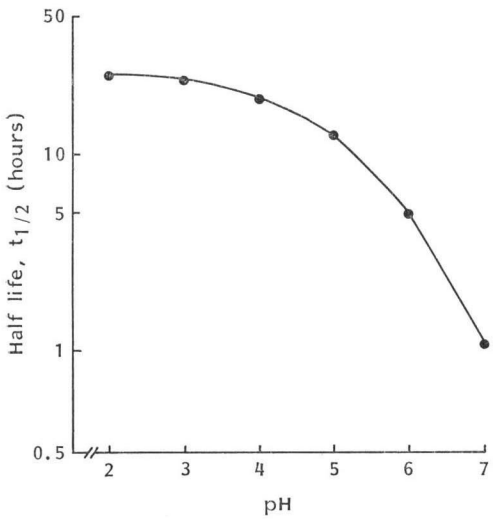
The absorption of KY-109 after oral administration of a dose equivalent to 25 mg/kg of KY-087 to

Table 3. Plasma levels of KY-087 after administration of KY-087 sodium, KY-106 and KY-109 at a dose equivalent to 25 mg/kg of KY-087 to rats.

Compound	Dose route	Vehicle	Plasma concentration (μg/ml)*						AUC (μg·hour/ml)	Bioavailability (%)
			7.5 minutes	15 minutes	30 minutes	60 minutes	120 minutes	240 minutes		
KY-087	iv	Saline	88.0±3.0	60.8±2.0	36.7±1.7	18.9±2.0	5.2±0.5	1.1±0.2	66.2±3.5	
KY-087	po	Distilled water	—	0.8±0.1	1.1±0.1	1.1±0.2	0.6±0.2	—	2.3±0.4	3.5
KY-106	po	1% Acacia (suspension)	—	0.4±0.2	0.9±0.04	0.8±0.05	—	—	1.0±0.02	1.5
KY-109	po	Distilled water	5.0±0.2	11.9±0.8	13.6±1.0	8.8±1.0	3.1±0.3	1.1±0.1	20.3±1.4	30.7

—: Not detected.
* : Mean±S.E. (n=5).

Fig. 1. Stability of KY-109 in aqueous solution at 35°C.



rats was compared with that of orally or intravenously administered KY-087 sodium and orally administered KY-106 (Table 3). KY-087 sodium and KY-106 when administered orally were poorly absorbed and the peak levels in plasma were 1.1 μg/ml and 0.9 μg/ml respectively, while KY-109 was absorbed rapidly and efficiently from the gastro-intestinal tract, the peak level being 13.6 μg/ml at 30 minutes. The bioavailability after oral administration (AUC_{po}/$AUC_{KY\text{-}087\ iv}$) was 30.7% for KY-109, compared with 3.5% for KY-087 sodium and 1.5% for KY-106.

Discussion

For the oral absorption of pro-drugs of β-lactam antibiotics in common with many other drugs, aqueous solubility and lipophilicity are known to be important factors[2]. In addition to having the desired lipophilicity and solubility properties, the pro-drug must possess an optimal lability. It must be stable in the gastro-intestinal lumen but should, upon absorption, be rapidly hydrolyzed to the parent drug.

In this study, KY-087 (the parent drug) is poorly absorbed orally in rats, probably due to a poor lipophilicity. KY-106 (the monofunctional pro-drug) in which only the strongly acidic 4-carboxyl group of KY-087 has been esterified has a very low solubility in water, although it has a high lipophilicity. Therefore, the incomplete absorption of KY-106 is probably attributed to its poor solubility in the gastro-intestinal fluids.

In contrast, KY-109 (the bifunctional pro-drug), in which, in addition to the 4-carboxyl ester, the side chain α-hydroxyl group has been esterified with L-alanine, containing a hydrophilic amino group, combines an increased lipophilicity with a good aqueous solubility. Furthermore, it is hydrolyzed to the parent drug in the presence of serum and intestinal homogenate. Thus, KY-109 was found to possess properties with regard to lipophilicity, aqueous solubility and hydrolysis in biological fluids that should make it an orally active pro-drug. When administered orally to rats, KY-109 was well absorbed, giving high blood levels of the parent drug. Although the exact mechanism of absorption of the bifunctional pro-drug has not been elucidated, it is reasonable to assume that, during or shortly after absorption of the pro-drug into the blood-stream from the gastro-intestinal tract both the L-alanyl and the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl residues are split off enzymatically with the formation of the parent drug.

Experimental

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. The NMR spectra were obtained on a Hitachi R-600 spectrometer. IR spectra were obtained in Nujol on a Shimadzu IR-400 spectrometer, and the main absorptions are given in cm^{-1}. Chemical analysis was performed by the microanalytical group of Kyoto University.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-(D-Mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl]-3-cephem-4-carboxylate (KY-106, **2**)

4-Bromomethyl-5-methyl-1,3-dioxol-2-one (15.5 g) was added to a stirred mixture of KY-087 (34 g)

and potassium acetate (7.0 g) in DMF (120 ml) at −20°C. After 1 hour, ethyl acetate (300 ml) was added to the reaction mixture and the obtained organic phase was washed with water followed by brine, and dried over Na_2SO_4. The filtered organic solution was evaporated *in vacuo* and the residue crystallized from methanol to give 22 g of KY-106 (**2**); mp 151°C (dec); IR (Nujol) 3350, 1820, 1780, 1730, 1680 cm^{-1}; NMR (acetone-d_6) δ 2.22 (s, 3H, dioxole-CH_3), 2.70 (s, 3H, thiadiazole-CH_3), 3.59, 3.97 (ABq, 2H, J=18 Hz, 2-CH_2), 4.07, 4.87 (ABq, 2H, J=14 Hz, 3-CH_2), 5.21 (d, 1H, J=6 Hz, α-CH), 5.15 (s, 2H, $COOCH_2$), 5.41 (d, 1H, J=6 Hz, α-OH), 5.12 (d, 1H, J=4.8 Hz, 6-H), 5.81 (dd, 1H, J= 9 and 4.8 Hz, 7-H), 7.42 (m, 5H, phenyl), 7.99 (d, 1H, J=9 Hz, CONH).

Anal Calcd for $C_{24}H_{22}N_4O_8S_3$: C 48.71, H 3.97, N 9.23, S 15.85.
Found: C 48.48, H 3.84, N 9.52, S 15.79.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-(1-Methyl-2-ethoxycarbonylvinyl)amino-3-[[5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate (**4**)

A mixture of 7-ACTD K (100 g) and ethyl acetoacetate (500 ml) was stirred at room temperature overnight. The resulting crystalline precipitate was filtered off, washed with ethyl acetate and added to a solution of 4-bromomethyl-5-methyl-1,3-dioxol-2-one (46 g) in DMF (400 ml) at −5~0°C. After stirring for 1 hour, aqueous methanol (10%, 440 ml) was added to the reaction solution, and stirring continued at room temperature for 30 minutes. The resulting precipitate was filtered off, washed with water followed by methanol and crystallized from dichloromethane - methanol to give 120 g of **4**; mp 150°C (dec); IR (Nujol) 1820, 1780, 1725, 1640, 1610 cm^{-1}; NMR (acetone-d_6) δ 1.16 (t, J=7 Hz, 3H, CH_3), 1.96 (s, 3H, CH_3C=), 2.19 (s, 3H, dioxole-CH_3), 2.67 (s, 3H, thiadiazole-CH_3), 3.60, 3.93 (ABq, J=18 Hz, 2H, 2-H_2), 4.00 (q, J=7 Hz, 2H, CH_2), 4.08, 4.73 (ABq, J=13 Hz, 2H, 3-CH_2S), 4.61 (s, 1H, C=CH), 5.13 (s, 2H, 4-$COOCH_2$), 5.18 (d, J=5.5 Hz, 1H, 6-H), 5.78 (dd, J=5.5 and 9 Hz, 1H, 7-H), 8.91 (d, J=9 Hz, 1H, =CNH).

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-Amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate (**5**)

Compound **4** (100 g) dissolved in dichloromethane (500 ml) was treated with methanol (25 ml) and conc HCl (30 ml) and stirred at 10~15°C for 4 hours. The resulting precipitate was filtered off, dissolved in water (500 ml) and washed with ethyl acetate. The aqueous layer was added to dichloromethane (500 ml) and $NaHCO_3$ (45 g) was added under stirring. The separated organic solution was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was crystallized from dichloromethane - ethyl acetate to yield 60 g of **5**; mp 108°C (dec); IR (Nujol) 3400, 3350, 1810, 1780, 1730 cm^{-1}; NMR (DMSO-d_6) δ 2.18 (s, 3H, dioxole-CH_3), 2.6 (br, 2H, NH_2), 2.67 (s, 3H, thiadiazole-CH_3), 3.47, 3.73 (ABq, 2H, J=18 Hz, 2-H_2), 4.04, 4.64 (ABq, 2H, J=14 Hz, CH_2S), 4.79 (d, 1H, J=5 Hz, 6-H), 4.97 (d, 1H, J=5 Hz, 7-H), 5.11 (s, 2H, dioxole-CH_2).

O-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]-D-mandelic Acid (**6**)

A solution of *N*-(*tert*-butoxycarbonyl)-L-alanine (95 g) and 4-dimethylaminopyridine (40 mg) in dichloromethane (400 ml) was added dropwise to a mixture of benzyl D-mandelate (121.0 g) and DCC (104 g) in dichloromethane (500 ml) at −5~0°C. The mixture was stirred at room temperature for 3 hours, filtered and concentrated *in vacuo*. The resulting solid was crystallized from petroleum ether to give 137.5 g of benzyl *O*-[*N*-(*tert*-butoxycarbonyl)-L-alanyl]-D-mandelate as a white crystalline solid. The latter was dissolved in ethyl acetate (300 ml) and hydrogenated over 5% Pd-C (13.8 g). The filtrate was concentrated and water (100 ml) and hexane (1,000 ml) were added to give 92 g of *O*-[*N*-(*tert*-butoxycarbonyl)-L-alanyl]-D-mandelic acid (**6**); mp 90.4°C; IR (Nujol) 3500, 3425, 1725, 1690 cm^{-1}; NMR ($CDCl_3$) δ 1.38 (s, 9H, $C(CH_3)_3$), 1.40 (d, 3H, J=7 Hz, CH_3CH), 4.40 (m, 1H, CH_3CH), 6.00 (s, 1H, PhC*H*), 7.2~7.5 (m, 5H, Ph), 9.27 (br s, 1H, NH).

Anal Calcd for $C_{16}H_{21}NO_6 \cdot H_2O$: C 56.30, H 6.79, N 4.10.
Found: C 56.44, H 6.77, N 4.29.

7-[D-*O*-[(*N*-*tert*-Butoxycarbonyl)-L-alanyl]mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]-methyl]-3-cephem-4-carboxylic Acid (**7**)

O-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]-D-mandelic acid (23.0 g) and triethylamine (9 ml) were dis-

solved in tetrahydrofuran (540 ml), and treated dropwise with ethoxycarbonylchloride (8.45 g) while stirring at −15°C. After 1 hour, a solution of 7-ACTD (23.3 g) and triethylamine (9.5 ml) in a mixture of tetrahydrofuran (300 ml) and water (200 ml) was added at −10°C, and the mixture stirred at 0°C for 1 hour and for a further hour at room temperature. The solvent was removed under reduced pressure and the residue was acidified with dilute H_3PO_4 and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 and evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography with chloroform - methanol (5: 1) to give 20 g of **7**; IR (Nujol) 3300, 1780, 1760, 1690 cm^{-1}; NMR (DMSO-d_6) δ 1.30 (d, 3H, J=7 Hz, $CHCH_3$), 1.35 (s, 9H, $C(CH_3)_3$), 2.67 (s, 3H, thiadiazole-CH_3), 3.45 (br s, 2H, 2-H_2), 3.91 ~ 4.75 (m, 4H, 3-CH_2S, COOH, $CHCH_3$), 4.96 (d, 1H, J=5 Hz, 6-H), 5.64 (dd, 1H, J=5 and 9 Hz, 7-H), 6.02 (s, 1H, $CHCONH$), 7.39 (s, 5H, Ph), 9.27 (d, 1H, J=9 Hz, CONH).

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-*O*-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]]mandelamido-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate (**8**)

Method A: KY-106 (2.75 g) and *N*-(*tert*-butoxycarbonyl)-L-alanine (0.97 g) were dissolved in dichloromethane (100 ml), and DCC (1.15 g) and 4-dimethylaminopyridine (60 mg) were added to the solution at 0°C. After stirring at the same temperature for 1 hour, the precipitated dicyclohexylurea (DCU) was removed. The filtrate was washed with 10% aqueous citric acid and brine, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was crystallized from methanol to give 3.3 g of the intermediate (**8**).

Method B: A solution of DCC (10.8 g) in dichloromethane (10 ml) was added dropwise to a solution of **5** (20.4 g) and **6** (17 g) in dichloromethane (160 ml) at −10 ~ 0°C. After stirring for 1 hour, the precipitated DCU was removed and the filtrate was evaporated *in vacuo*. The residue was crystallized from methanol to give 21.2 g of the intermediate (**8**).

Method C: A solution of 4-bromomethyl-5-methyl-1,3-dioxol-2-one (2.33 g) in DMF (15 ml) was added to a mixture of **7** (6.5 g) and potassium acetate (0.98 g) at −10°C. After stirring for 30 minutes, water (100 ml) was added and the reaction mixture was extracted with ethyl acetate (2 × 50 ml). The combined oragnic layers were washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was crystallized from methanol to give 6.2 g of the intermediate (**8**); mp 156°C (dec); IR (Nujol) 3340, 1820, 1780, 1750, 1680 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (d, 3H, J=7 Hz, $CHCH_3$), 1.41 (s, 9H, $C(CH_3)_3$), 2.22 (s, 3H, dioxole-CH_3), 2.73 (s, 3H, thiadiazole-CH_3), 3.42, 3.82 (ABq, 2H, J=17 Hz, 2-H_2), 3.89, 4.91 (ABq, 2H, J=14 Hz, 3-CH_2S), 4.1 ~ 4.55 (m, 1H, $CHCH_3$), 5.0 (br, 1H, NH), 5.03 (d, 1H, J=5 Hz, 6-H), 5.05 (s, 2H, dioxole-CH_2), 5.69 (dd, 1H, J=5 and 9 Hz, 7-H), 6.19 (s, 1H, $CHCONH$), 7.4 (s, 5H, Ph), 7.68 (d, 1H, J=9 Hz, CONH).

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-*O*-(L-Alanyl)mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate Hydrochloride (KY-109, **3**)

11.1 N HCl - methanol (12.5 ml) was added dropwise to a solution of **8** (21.2 g) in acetone (170 ml) at 5°C. After stirring overnight at room temperature, the resulting crystalline precipitate was filtered off, and recrystallized from ethanol to give 19 g of KY-109 (**3**); mp 158°C (dec); IR (Nujol) 1820, 1780, 1740, 1690 cm^{-1}; NMR (DMSO-d_6) δ 1.48 (d, 3H, J=7 Hz, $CHCH_3$), 2.18 (s, 3H, dioxole-CH_3), 2.66 (s, 3H, thiadiazole-CH_3), 3.62 (br s, 2H, 2-H_2), 4.2 (m, 1H, $CHCH_3$), 4.07, 4.65 (ABq, 2H, J=14 Hz, 3-CH_2S), 5.05 (d, 1H, J=5 Hz, 6-H), 5.15 (s, 2H, dioxole-CH_2), 5.72 (dd, 1H, J=5 and 9 Hz, 7-H), 6.13 (s, 1H, $CHCONH$), 7.4 (m, 5H, Ph), 8.73 (br, 3H, NH_3^+), 9.46 (d, 1H, J=9 Hz, CONH).

Anal Calcd for $C_{27}H_{28}N_5O_9S_3Cl$: C 46.45, H 4.04, N 10.03, S 13.78.
Found: C 46.53, H 4.02, N 10.02, S 13.75.

Test Antibiotics

Cefamandole, cephalexin and cefaclor (Shionogi & Co., Ltd.) were commercial products. KY-087 sodium was prepared using the method described by HOOVER[1].

Determination of MICs

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method, using Mueller-Hinton agar (MHA). Serial two-fold dilutions of freshly prepared antibiotic solutions were

mixed with melted MHA in Petri dishes. Plates were inoculated with one loopful of 10^{-2} fold diluted overnight culture of the test organisms in Mueller-Hinton broth (MHB) unless otherwise described. The MIC values (μg/ml) were determined after 18 hours of incubation at 37°C.

Absorption Studies

Male Wistar strain rats weighing 180~220 g were used. Before the experiment, the animals were starved overnight but were allowed to drink water. KY-087 was administered orally or intravenously as an aqueous solution of the sodium salt, at a dose 25 mg/kg, to the test animals. KY-106 and KY-109 were given orally at a dose equivalent to 25 mg/kg of KY-087 as an aqueous suspension and an aqueous solution, respectively.

Blood samples were collected from the jugular vein at specified intervals after administration. The antibiotic concentrations in the plasma were bioassayed using standard solutions prepared with control plasma of rats. Bioassay was performed by the agar-well method with *B. subtilis* as the test organism.

Aqueous Solubility

The aqueous solubilities of the compounds were estimated at 25°C by shaking an excess of each with 10 ml of water, for a time period limited to 3 hours to minimize degradation of the compounds. The saturated solutions were filtered, diluted, and the concentrations of the compounds measured by the high-performance liquid chromatographic (HPLC) method (Waters ALC/GPC conpact type, model 440 detector, 245 nm filter, model 45 pump, model WISP 710B injector, and reverse phase Radial Pac μ-Bondapac C column). Elution was carried out with aqueous KH_2PO_4 (0.3%) - CH_3CN (80 : 20 for KY-087, and 60 : 40 for KY-109 and KY-106) at a flow rate of 0.3 ml/minute.

Partition Coefficient

Solution of 1.5×10^{-2} mM of a compound was prepared in 100 ml of 1/15 M phosphate buffer (pH 6.5) saturated with 1-octanol. The solution (10 ml) was put into a test tube containing 1-octanol (5 ml) saturated with pH 6.5 buffer. The test tube was shaken vigorously at 25°C. When equilibrium had been achieved (1 hour), the aqueous layer was separated by centrifugation, and the concentration of the drug was measured by HPLC method similar to that described for the aqueous solubility.

In Vitro Hydrolysis

In Buffer Solutions: KY-109 was added to an appropriate buffer solution (pH 2~6, 0.1 M citrate; pH 6~7, 0.1 M phosphate) at 50 μg/ml, and the solutions were maintained at 35°C. Samples were withdrawn at proper intervals and the remaining intact KY-109 was determined by the HPLC method (Shimadzu LC-3A, SPP-2A detector, 270 nm filter and reverse phase Unisil Q C_{18} column). Elution was carried out with aqueous 0.05 M KH_2PO_4 - CH_3CN (60: 40) at a flow rate of 2.0 ml/minute.

In Intestine Homogenate: The small intestine was obtained from a freshly killed rat and was immediately thoroughly washed with cold saline to remove any contents. It was homogenized at 31.5% w/v in ice-cold saline, using an Ultra Disperser (LK-21, Yamato). KY-109 was dissolved in saline at a concentration equivalent to 200 μg/ml of the parent cephalosporin. The solution (1 ml) was rapidly added to the intestinal homogenate (19 ml) to give a reaction mixture with KY-109 at final concentration of 10 μg/ml in 30% w/v homogenate that was incubated at 37°C and sampled at 2, 5, 15, 30 and 60 minutes after mixing. Samples of 3 ml were poured into a mixture of pH 7.4 phosphate buffer (1 ml) and dichloromethane (3 ml) and shaken vigorously. The aqueous phase was separated and assayed for the resulting parent compound by the agar-well method with *B. subtilis* ATCC 6633 as the test organism.

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