ORALLY ACTIVE PIVALOYLOXYMETHYL 3-(ISOXAZOLIDIN-5-YL)CEPHALOSPORINS†

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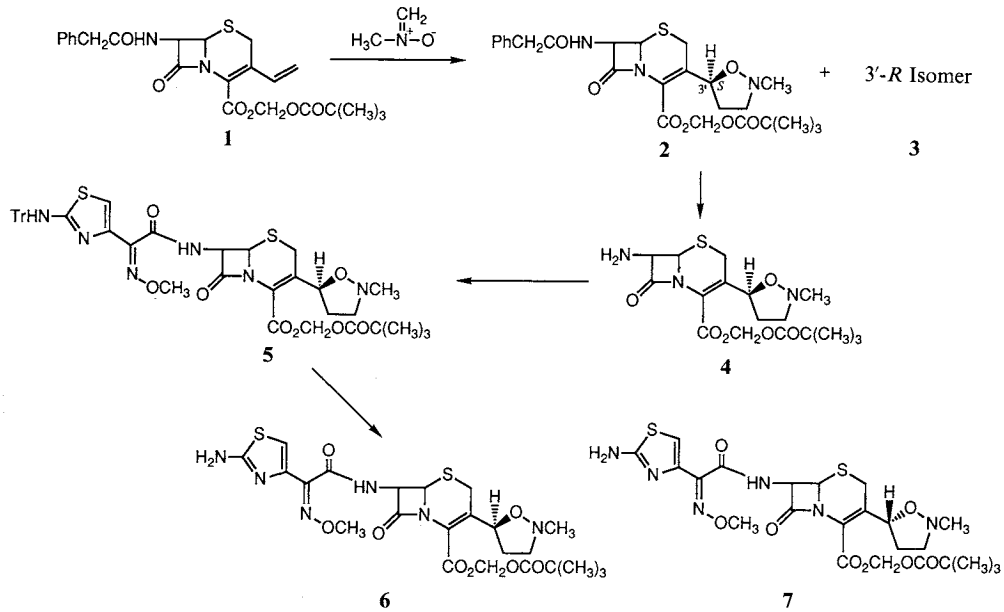
Many of potent cephalosporins with aminothiazolyl-oxyimino groups in the C-7 side chain generally show the poor absorption by oral administration. To increase the intestinal absorption, esterifications of the carboxylic acid in cephem have been achieved. The esters of prodrugs are mostly diacylacetals of formaldehyde or acetaldehyde.[1] These esters are rapidly hydrolyzed by esterase to give the active parent antibiotic after absorption from the intestinal tract.

In the preceding paper, we have described the syntheses and antibacterial activities of new 3-(isoxazolidin-5-yl)cephalosporins having aminothiazolyl-oxyimino side chains.[2] Most of the compounds were converted to prodrug-esters, which were evaluated the absorption by oral administration. This report describes the syntheses and biological activities of pivaloyloxymethyl 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*S*)- and (*R*)-2-methylisoxazolidin-5-yl]-3-cephem-4-carboxylates (**6** and **7**).

The reaction of pivaloyloxymethyl 7-phenylacetamido-3-vinyl-3-cephem-4-carboxylate (**1**) with *N*-methylnitrone, generated from 38% formalin and *N*-methylhydroxylamine hydrochloride in the presence of sodium acetate *in situ*, at 90°C gave two diastereomeric 3-(2-methylisoxazolidin-5-yl)-3-cephems (**2** and **3**) in 78% yield (Fig. 1). These isomers were isolated on silica gel chromatography. 3′-(*S*)-Isomer **2** was treated with phosphorous pentachloride, pyridine and methanol to give 7-amino derivative **4**. The amino group of **4** was acylated with (*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetic acid by *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBT) method to give **5** in good yield. The

Fig. 1. Synthesis of orally active 3-isoxazolidine cephalosporins.



† Dedicated to the late Professor HAMAO UMEZAWA on the occasion of the 30th anniversary of the Institute of Microbial Chemistry.

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treatment of **5** with 50% formic acid gave a final product **6**. 3'-(*R*)-Isomer **3** was also derivatized to **7** by a similar method.

The parent antibiotics of **6** and of **7** showed good antibacterial activities.[2] The results of urinary recovery of **6**, **7** and cefteram pivoxil (CFTM-PI) after oral administration in mice are shown in Table 1. Although the parent compounds of **6** and of **7** showed negligible recoveries (2.2 and 1.4%, respectively), esters **6** and **7** were well absorbed from the intestinal tract and recovered in urine. According to the results of *in vitro* antibacterial activities and urinary recoveries, **6** was biologically further evaluated. Pharmacokinetic parameters and *in vivo* antibacterial activities of **6** and CFTM-PI after oral administration in mice are listed in Table 2. On the half life and AUC, **6** was superior to CFTM-PI. ED_{50} values of **6** against pathogenic *Staphylococcus aureus* and *Escherichia coli* are smaller than those of CFTM-PI. The parent compound of **6** showed a low binding (6%) to the human serum albumin and it caused no death at 3 g/kg after intravenous administration into mice.

Pivaloyloxymethyl 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*S*)-2-methylisoxazolidin-5-yl]-3-cephem-4-carboxylate (**6**) prepared here shows a good absorption from the intestinal tract and a good activity *in vivo* against systemic pathogen in mice.

Table 1. Urinary recovery of **6**, **7** and CFTM-PI.

Compound	Recovery (%)[a]
6	52
7	49
CFTM-PI	37

[a] Dose: 15 mg/kg (po).

Experimental

Pivaloyloxymethyl 7-Phenylacetamido-3-vinyl-3-cephem-4-carboxylate (**1**)

To a solution of *p*-methoxybenzyl 7-phenylacetamido-3-vinyl-3-cephem-4-carboxylate[3] (464 mg) in dichloromethane (2 ml) were added anisole (1 ml) and trifluoroacetic acid (2 ml) at 5°C. After 1 hour, the solution was concentrated. The residue was triturated with isopropyl ether. The obtained solid was suspended in water and pH of the mixture was adjusted to 7 with aq $NaHCO_3$ solution. The resulting aq solution was lyophilized to give sodium salt (362 mg). To a solution of sodium salt (362 mg) in DMF (2 ml) was dropwise added iodomethyl pivalate (363 mg) at −10°C. After stirring for 15 minutes at the same temperature, the mixture was poured into ice-water and extracted with EtOAc. The organic layer was washed with satd NaCl solution, dried over anhydr Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with $CHCl_3$ to give **1** (330 mg). ^1H NMR (90 MHz, $CDCl_3$) δ 1.25 (9H, s, $C(CH_3)_3$), 3.43 (1H, d, $J=18$ Hz, 2-Hb), 3.63 (2H, s, CH_2Ar), 3.66 (1H, d, $J=18$ Hz, 2-Ha), 4.97 (1H, d, $J=5$ Hz, 6-H), 5.36 (1H, d, $J=12$ Hz, CH=CH_2), 5.50 (1H, d, $J=18$ Hz, CH=CH_2) and 7.06 (1H, dd, $J=12$ and 18 Hz, $CH=CH_2$).

Pivaloyloxymethyl 7-Phenylacetamido-3-[(*S*)-2-methylisoxazolidin-5-yl]-3-cephem-4-carboxylate (**2**) and Pivaloyloxymethyl 7-Phenylacetamido-3-[(*R*)-2-methylisoxazolidin-5-yl]-3-cephem-4-carboxylate (**3**)

To a solution of **1** (687 mg), sodium acetate (185 mg) and 38% formalin (0.24 ml) in a mixture of dioxane (9 ml) and ethanol (4.5 ml) was dropwise added a solution of *N*-methylhydroxylamine hydrochloride (188 mg) in 83% aq ethanol (5.5 ml) at

Table 2. Pharmacokinetic parameters in mice and *in vivo* activity (ED_{50}) against systemic infections in mice.

Compound	Pharmacokinetic parameter[a]			ED_{50} (mg/kg)[b,c]		
	$T_{1/2}$ (hour)	AUC (μg·hours/ml)	$C_{max\ ob}$ (μg/ml)	*S.a.*	*E.c.*	*K.p.*
6	0.44	15.7	9.9	23	2.5	17
CFTM-PI	0.24	6.96	9.4	>50	8.0	9.5

[a] Dose: 15 mg/kg (po).

[b] Mice (ICR male, 4 weeks) were treated with the test-compound (po) 1 hour after infection. Infecting dose (ip): 9.3×10^6 cfu/mouse for *S.a.* (*Staphylococcus aureus* Smith), 2.6×10^7 for *E.c.* (*Escherichia coli* No. 29) and 1.3×10^5 for *K.p.* (*Klebsiella pneumoniae* PCI 602).

[c] MICs of parent compound of **6**: *S.a.* 1.56 (μg/ml), *E.c.* 0.025 and *K.p.* 0.025. MICs of CFTM: *S.a.* 0.78, *E.c.* 0.025 and *K.p.* 0.012.

room temperature. After stirring at 90°C for 1 hour, the solution was concentrated. The residue was triturated with dichloromethane and the organic layer was washed with aq $NaHCO_3$ solution and satd NaCl solution, dried over anhydr Na_2SO_4 and evaporated to give a solid. The obtained solid was chromatographed on silica gel with chloroform - methanol (997 : 3) to give **2** (565 mg) and **3** (155 mg). **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (9H, s, $C(CH_3)_3$), 2.07 and 2.53 (each 1H, m, 3-Hb and 4-Hb of isoxazolidine), 2.68 (3H, br s, NCH_3), 2.84 and 3.27 (each 1H, m, 3-Ha and 4-Ha of isoxazolidine), 3.61 (1H, d, J=16 Hz, one of CH_2Ar), 3.62 (1H, d, J=19 Hz, 2-Hb), 3.68 (1H, d, J=16 Hz, one of CH_2Ar), 3.79 (1H, d, J=19 Hz, 2-Ha), 4.92 (1H, d, J=5 Hz, 6-H), 5.20 (1H, br t, J=8 Hz, 3'-H), 5.80 (1H, d, J=5 Hz, one of CO_2CH_2O), 5.82 (1H, dd, J=5 and 9 Hz, 7-H), 5.89 (1H, d, J=5 Hz, one of CO_2CH_2O) and 6.00 (1H, d, J=9 Hz, CONH). **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (9H, s, $C(CH_3)_3$), 2.00, 2.47 and 2.68 (each 1H, m, 3-Hb, 4-Hb and 4-Ha of isoxazolidine), 2.70 (3H, br s, NCH_3), 3.22 (1H, m, 3-Ha of isoxazolidine), 3.57 (1H, d, J=19 Hz, 2-Hb), 3.62 (1H, d, J=16 Hz, one of CH_2Ar), 3.66 (1H, d, J=19 Hz, 2-Ha), 3.68 (1H, d, J=16 Hz, one of CH_2Ar), 4.93 (1H, d, J=5 Hz, 6-H), 5.57 (1H, br t, J=7 Hz, 3'-H), 5.75 (1H, dd, J=5 and 9 Hz, 7-H), 5.84 (1H, d, J=5 Hz, one of CO_2CH_2O), 5.87 (1H, d, J=5 Hz, one of CO_2CH_2O) and 6.07 (1H, d, J=9 Hz, CONH).

Pivaloyloxymethyl 7-[(*Z*)-2-(2-Tritylaminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*S*)-2-methyl-isoxazolidin-5-yl]-3-cephem-4-carboxylate (**5**)

Anhydr pyridine (119 mg) was added to a mixture of PCl_5 (312 mg) in dichloromethane (4 ml) at 0°C. After stirring at the same temperature for 1 hour, compound **2** (259 mg) was added to above solution at 8°C. The stirring was continued for 1.5 hours at 8°C. The mixture was cooled to −30°C and methanol (2 ml) was added. After 1.5 hours below −15°C, it was diluted with dichloromethane (15 ml) and extracted with satd NaCl solution (15 ml). The aq layer was adjusted to pH 9 with aq $NaHCO_3$ solution and extracted with chloroform. After evaporation, **4** (166 mg) was obtained. To a solution of **4** (100 mg) in DMF (2 ml) were added (*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetic acid (111 mg), DCC (57 mg) and HOBT (37 mg). After stirring for 2 hours at room temperature, the solution was concentrated. The residue was dissolved in EtOAc and the solution was washed with satd NaCl solution, dried over anhydr Na_2SO_4 and concentrated. The solid was chromatographed on silica gel with chloroform to give **5** (182 mg, 88%).

Pivaloyloxymethyl 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*S*)-2-methyl-isoxazolidin-5-yl]-3-cephem-4-carboxylate (**6**)

A solution of **5** (200 mg) in 50% formic acid (2 ml) was stirred at room temperature for 1.5 hours. After filtration and evaporation, the residue was dissolved in chloroform. The solution was washed with aq $NaHCO_3$ solution and satd NaCl solution, dried over anhydr Na_2SO_4 and evaporated to afford a solid. The obtained solid was chromatographed on silica gel with chloroform - MeOH (49 : 1) to give **6** (130 mg, 92%). FAB-MS *m/z* 583 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (9H, s, $C(CH_3)_3$), 2.15 (1H, m, 4-Hb of isoxazolidine), 2.56 (1H, m, 3-Hb of isoxazolidine), 2.70 (3H, s, NCH_3), 2.87 (1H, m, 4-Ha of isoxazolidine), 3.30 (1H, m, 3-Ha of isoxazolidine), 3.69 (1H, d, J=19 Hz, 2-Hb), 3.88 (1H, d, J=19 Hz, 2-Ha), 4.07 (3H, s, OCH_3), 5.05 (1H, d, J=5 Hz, 6-H), 5.26 (1H, m, 3'-H), 5.85 and 5.90 (each 1H, d, J=5 Hz, CO_2CH_2O), 6.0 (1H, dd, J=5 and 9 Hz, 7-H), 6.89 (1H, s, 5-H of thiazole) and 7.40 (1H, d, J=9 Hz, CONH).

Pivaloyloxymethyl 7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*R*)-2-methyl-isoxazolidin-5-yl]-3-cephem-4-carboxylate (**7**)

Compound **7** was synthesized from **3** by similar procedures used for **6**. FAB-MS *m/z* 583 (MH^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (9H, s, $C(CH_3)_3$), 2.06 (1H, m, 4-Hb of isoxazolidine), 2.50 (1H, m, 3-Hb of isoxazolidine), 2.73 (3H, s, NCH_3), 3.27 (1H, m, 3-Ha of isoxazolidine), 3.67 (1H, d, J=19 Hz, 2-Hb), 3.80 (1H, d, J=19 Hz, 2-Ha), 4.09 (3H, s, OCH_3), 5.06 (1H, d, J=5 Hz, 6-H), 5.64 (1H, m, 3'-H), 5.93 (3H, m, 7-H and CO_2CH_2O), 6.99 (1H, s, 5-H of thiazole) and 7.20 (1H, d, J=9 Hz, CONH).

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