SYNTHESIS AND ORAL ACTIVITY OF ME1207, A NEW ORALLY ACTIVE CEPHALOSPORIN

Sir:

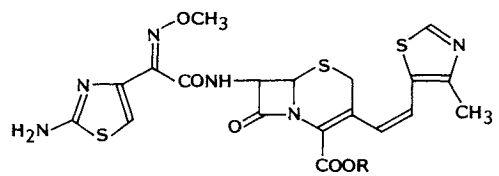
The need still exists for the development of new orally active, semi-synthetic cephalosporins which exhibit potent, broad-spectrum, antibiotic activity. In a previous paper[1] relating to the antibacterial activity and oral absorption of 3-alkylthio-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(*O*-substituted oxyiminoacetamido]cephalosporins, we reported that the pivaloyloxymethyl ester of 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methylthio-3-cephem-4-carboxylic acid showed high urinary recovery after oral administration to mice. The free acid, active form of this cephalosporin, showed excellent activity against Gram-negative bacteria, but did not show satisfactory activity against Gram-positive bacteria. We then looked for improvement of the antibacterial activity against Gram-positive bacteria by modification of the 3-substituent while retaining the high antibacterial activity against Gram-negative bacteria. As a result, we found that a new orally active cephalosporin, pivaloyloxymethyl 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3(*Z*)-(4-methylthiazol-5-yl)vinyl-3-cephem-4-carboxylate (**9**, ME1207)[2] showed excellent oral activity and ME1206 (**8**)[2], the active form of ME1207, showed a potent and broad antibacterial activity against Gram-positive and Gram-negative bacteria. Herein reported is the synthesis and biological activity of ME1207 and ME1206.

ME1207 was synthesized as outlined in Scheme 1. The *p*-methoxybenzyl 7-phenylacetamido-3-chloromethyl-3-cephem-4-carboxylate[3] (**1**) was converted to the corresponding iodide by treatment with NaI in acetone, which was, without purification, treated with PPh_3 to give the triphenylphosphonium iodide (**2**) in 90% overall yield. Wittig reaction of **2** with 5-formyl-4-methylthiazole (**3**) was carried out in dichloromethane-water at room temperature in the presence of sodium bicarbonate to give 84% yield of the vinyl derivative (**4**) in a form of a 4.7 : 1 mixture of the *Z* (*cis*) and *E* (*trans*) isomers. The structure of the major product having a smaller vinyl coupling constant ($J=11$ Hz) in the ^{1}H NMR spectra was determined to *Z*, whereas the minor one having the larger coupling constant ($J=16$ Hz) was *E* isomer. Since it was difficult to separate these isomers by column chromatography in this stage, the product was used for the next step without separation of the isomers. After the removal of 7-*N*-phenylacetyl substituent of **4** by standard method (PCl_5 and then MeOH), 7-aminocephem esters (**5**) was coupled with 2-(2-tritylaminothiazol-4-yl)-2(*Z*)-methoxyiminoacetic acid (**6**) using $POCl_3$ as a coupling reagent ($POCl_3$ - pyridine, -20°C, 1 hour) to give the protected derivatives (**7**) in 47% overall yield from **4**. Removal of the protective groups of **7** with CF_3COOH - anisole (5°C, 1 hour) afforded the free acid. After neutralization, its sodium salt was purified by Diaion HP-20 column chromatography and followed by crystallization from water to give new cephalosporin (Na salt of **8**, ME1206) as a single *Z*-isomer. The sodium salt of **8** was treated with iodomethyl pivalate in DMF (-20°C, 1 hour) to give the pivaloyloxymethyl ester (**9**, ME1207) in 73% yield. Physico-chemical properties of ME1206 and ME1207 are summarized in Table 1.

The MICs of ME1206 were determined by the standard, 2-fold agar dilution method. In Table 2, the MICs of ME1206 against several microorganisms are summarized and compared with those values for cefixime (CFIX)[4], cefteram (CFTM)[5] and cefaclor (CCL)[6]. ME1206 showed a potent and broad antibacterial activity against both Gram-positive and Gram-negative bacteria. Especially, the activity of ME1206 against Gram-positive bacteria was the most potent. The activity of ME1206 against Gram-negative bacteria was more potent than that of CCL and comparable with those of CFIX and CFTM.

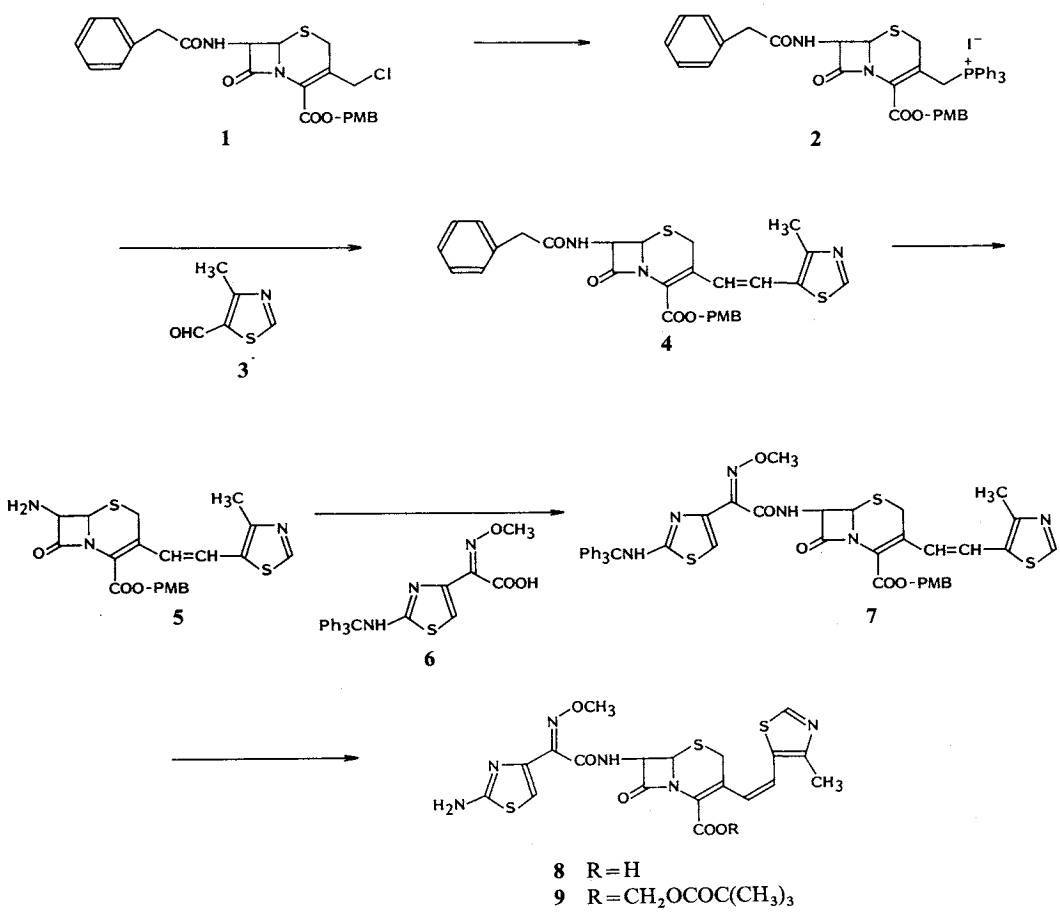
The urinary recovery and the ED_{50} values of ME1207, cefteram pivoxil (CFTM-PI)[4], CFIX and CCL in mice are shown in Table 3. Urinary recovery was determined by disk method on Sensitivity test agar (Nissui) using *Escherichia coli* K-12 HW 8236 as a test strain after oral administration of ME1207, CFTM-PI, CFIX and CCL (25 mg/kg as a parental cephalosporin) in mice ($n=3$, $0\sim4$ hours). ME1207 showed high urinary recovery. The *in vivo*

Fig. 1. ME1207 and ME1206.



ME1206 R=H
ME1207 R=$CH_2OCOC(CH_3)_3$

Scheme 1.



PMB: *p*-Methoxybenzyl group.

Table 1. Physico-chemical data of ME1206 and ME1207.

	ME1206	ME1207
MP (°C)	195~200 (dec)	127~129
IR (KBr) cm^{-1}	3450, 1775, 1680, 1620, 1590	3450, 1790, 1760, 1680, 1620
SI-MS (*m/z*) $(M+H)^+$	529	621
$[\alpha]_D^{20}$	+121.6° (*c* 0.5, H_2O)	−48.5° (*c* 0.5, MeOH)
^1H NMR 270 MHz	(DMSO-d_6) δ 2.30 (3H, s, CH_3), 3.00, 3.28 (2H, ABq, J=18 Hz, 2-H), 3.82 (3H, s, OCH_3), 5.10 (1H, d, J=5 Hz, 6-H), 5.62 (1H, dd, J=5, 8 Hz, 7-H), 6.34 (1H, d, J=11 Hz, CH=), 6.71 (1H, s, thiazole 5-H), 6.77 (1H, d, J=11 Hz, CH=), 7.22 (2H, br s, NH_2), 8.89 (1H, s, thiazole 2-H), 9.54 (1H, d, J=8 Hz, NH)	($CDCl_3$) δ 1.15 (9H, s, $C(CH_3)_3$), 2.44 (3H, s, CH_3), 3.30, 3.53 (2H, ABq, J=18.7 Hz, 2-H), 4.03 (3H, s, OCH_3), 5.21 (1H, d, J=5 Hz, 6-H), 5.52 (2H, br s, NH_2), 5.79, 5.85 (2H, ABq, J=5.5 Hz, $-CH_2$), 6.11 (1H, dd, J=5, 8 Hz, 7-H), 6.37 (1H, d, J=11.7 Hz, CH=), 6.67 (1H, d, J=11.7 Hz, CH=), 6.80 (1H, s, thiazole 5-H), 7.93 (1H, d, J=8 Hz, NH), 8.58 (1H, s, thiazole 2-H)

antibacterial activity were also tested using mice infected with Gram-positive and Gram-negative organisms. The mice were challenged intraperitoneally with 10^7 to 10^8 cfu/mouse of the bacteria. The animals were treated orally with the cephalosporins at 1 hour after infection. The number of surviving

Table 2. *In vitro* activity of ME1206 and related antibiotics.

Test organisms	ME1206	CFIX	CFTM	CCL
	MICs (μg/ml)			
Staphylococcus aureus 606[a]	0.78	6.25	6.25	3.13
S. aureus 606 E-25	0.78	6.25	3.13	3.13
S. aureus 209P JC-1	0.78	12.5	3.13	1.56
S. aureus Smith (1)	0.39	12.5	3.13	0.78
Bacillus subtilis ATCC 6633	0.20	50	0.78	0.20
Escherichia coli W3630 RGN823[a]	0.39	0.78	0.39	25
E. coli NIHJ JC-2	0.39	0.39	0.78	12.5
E. coli No. 29	0.39	0.20	0.39	1.56
Klebsiella pneumoniae GN69[a]	0.20	0.05	0.20	3.13
K. pneumoniae PCI602	0.39	0.10	0.39	0.78
Salmonella typhi 0-901-W	0.05	<0.025	0.05	0.78
S. typhimurium LT-2	0.39	0.05	0.39	1.56
Shigella dysenteriae (shiga)	0.05	0.39	0.05	0.78
Escherichia coli 255[b]	25	>100	12.5	>100
E. coli 255/S-1	0.39	0.78	0.39	1.56
Proteus vulgaris GN76[b]	0.20	<0.025	0.20	>100
P. vulgaris GN76/C-1[b]	0.20	0.05	3.13	>100
Morganella morganii 1510[b]	12.5	50	25	>100
M. morganii 1510/S-1	0.20	0.39	0.20	6.25
Providencia rettgeri GN624[b]	3.13	0.78	6.25	>100
P. rettgeri J-0026	1.56	0.20	1.56	100
Enterobacter cloacae GN7471[b]	25	50	50	>100
E. cloacae G-0008[b]	0.78	0.78	1.56	>100
Serratia marcescens GN10857[b]	12.5	25	100	>100
S. marcescens GN629[b]	1.56	0.78	3.13	>100
Pseudomonas aeruginosa GN10362[b]	25	100	>100	>100
P. aeruginosa MB-3833	12.5	50	100	>100

[a] Penicillinase producing strain.
[b] Cephalosporinase producing strain.

Table 3. Protective effect (ED_{50}, mg/mouse) of ME1207 and related antibiotics against experimental infection of mice and urinary recovery (mouse, $n=3$, 0~4 hours, %).

Organism	Challenge dose (cfu/mouse)		ME1207	CFTM-PI	CFIX	CCL
Staphylococcus aureus MS16040	4.3×10^8 ($48LD_{50}$)	ED_{50} (mg/mouse)	0.33	>2.0	NT	>2.0
		MIC (μg/ml)	0.78	3.13	NT	3.13
Escherichia coli No. 29	3.0×10^7 ($37LD_{50}$)	ED_{50} (mg/mouse)	0.027	0.032	0.032	0.040
		MIC (μg/ml)	0.39	0.39	0.20	1.56
Urinary recovery (%)			21.6	28.0	10.5	53.5

mice was recorded 1 week after infection (n=8 in a group). The data of the ED_{50} (mg/mouse) values of the cephalosporins against *Staphylococcus aureus* MS16040 and *E. coli* No. 29 are shown in Table 3. ME1207 was more effective than CCL and CFTM-PI against *S. aureus* MS16040. Against

E. coli No. 29, it was comparable in activity to CFIX and CFTM-PI.

These data indicate that pivaloyloxymethyl 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3(*Z*)-(4-methylthiazol-5-yl)vinyl-3-cephem-4-carboxylate (ME1207) is a new orally active cephalosporin. Clinical evaluation of ME1207 is being pursued.

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