STUDIES ON β-LACTAM ANTIBIOTICS

IX.† SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW ORALLY ACTIVE CEPHALOSPORIN, CEFIXIME (FK027)

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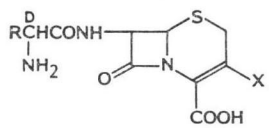
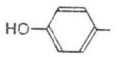
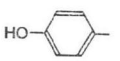
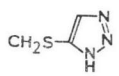
The synthesis and some biological properties of 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic acid (**3**, FK027)** are described. Diphenylmethyl 7-amino-3-vinyl-3-cephem-4-carboxylate hydrochloride (**8**), the cephem precursor to FK027 was prepared from 7-aminocephalosporanic acid (7-ACA) by two parallel routes differing primarily in the protection of the 7-amino group. Compound **8** was alternatively prepared from deacetylcephalosporin C sodium salt (DCCNa) with improved yields. Two pathways for the conversion of **8** to FK027 are provided. The new orally active cephalosporin, FK027, possesses a widely expanded antimicrobial activity and high stability to β-lactamases.

During the past decade remarkable improvements have been made among the injectable β-lactam antibiotics such as cephalosporins,[1~3] cephamycins,[4,5] oxacephamycin,[6] and penicillins.[7] In contrast to these developments, progress has been less evident among the oral β-lactam antibiotics such as the cephalosporins. Only cephalexin-like analogs (Fig. 1) have become available since cephalexin was brought to the market. This unique pharmacological property of oral absorption has been thought to be mainly related to the presence of the phenylglycyl or related moieties at the 7-position, although it is under the influence of a substituent group at the 3-position.[8]

Fig. 1. Structures of cephalexin-like analogs.



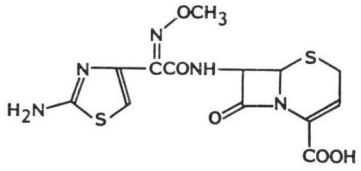
R	X	Generic name
	CH_3	Cephalexin
	Cl	Cefaclor
	CH_3	Cephradine
	OCH_3	Cefroxadine
	CH_3	Cefadroxil
		Cefatrizine

Since ceftizoxime (**1**) (Fig. 2)[9~12] with widely expanded antimicrobial activities and high stability to β-lactamases was found in our laboratories, we have intensively focused our attention on searching for a new orally active cephalosporin possessing the same antimicrobial activity as ceftizoxime.

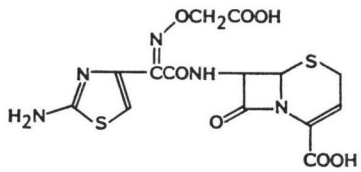
During the course of our research on ceftizoxime and related compounds, we found a new orally active cephalosporin (**2**, Fig. 2). As a

† Paper VIII. See ref 13).
** Generic name: Cefixime.

Fig. 2. Structures of ceftizoxime (**1**) and **2**.

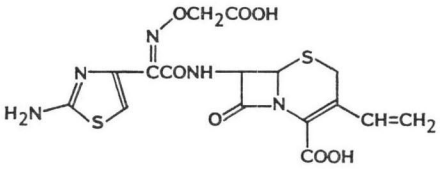


Ceftizoxime (**1**)



2

Fig. 3. Structure of cefixime (FK027).



3

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic acid.

result of modification of the aryl moiety in the 7-acyl side chain[13] and the C-3 substituent in **2**,[14] we selected FK027 as a candidate for human trials.

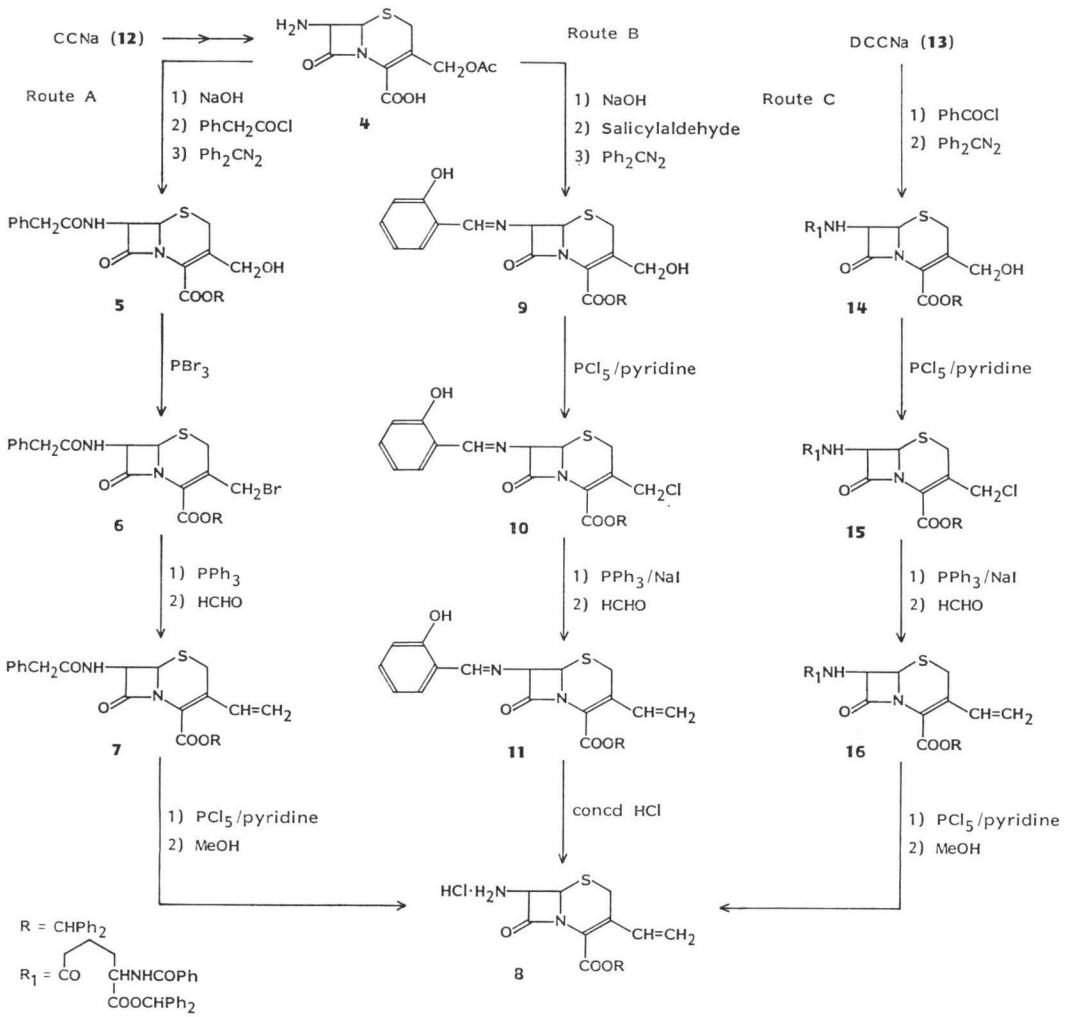
We here wish to report the synthesis and some biological properties of FK027 (**3**) (Fig. 3), which is structurally distinct from cephalexin analogs.

Chemistry

Three synthetic routes to diphenylmethyl 7-amino-3-vinyl-3-cephem-4-carboxylate hydrochloride (**8**)[15] are displayed in Scheme 1. Routes A and B show the synthesis of **8** from 7-aminocephalosporanic acid (7-ACA, **4**). In route A, diphenylmethyl 3-hydroxymethyl-7β-phenylacetamido-3-cephem-4-carboxylate (**5**) was obtained by alkaline hydrolysis of **4** followed by phenylacetylation and esterification, as reported in a previous paper.[16] The hydroxymethyl group at the 3-position of **5** was brominated with phosphorous tribromide to afford **6**. Reaction of **6**, first with triphenylphosphine (PPh_3), and then formaldehyde under aqueous alkaline conditions gave the 3-vinylcephem (**7**). The phenylacetyl side chain of **7** was cleaved by the known imino-chloride method[17] to afford diphenylmethyl 7-amino-3-vinyl-3-cephem-4-carboxylate hydrochloride (**8**). In route B, the *o*-hydroxybenzylidene group was introduced as a protective group of the amino function being easily cleaved by acid treatment. The 3-hydroxymethyl derivative (**9**) was prepared from **4** by alkaline hydrolysis under mild conditions followed by treatment with salicylaldehyde and diphenyldiazomethane. The hydroxymethyl group at the 3-position of **9** was chlorinated with phosphorus pentachloride (PCl_5) to give the 3-chloromethyl derivative (**10**). **10** was treated with sodium iodide and PPh_3 in *N*,*N*-dimethylformamide (DMF) to afford the corresponding phosphonium salt. The phosphonium salt was converted to the 3-vinyl derivative (**11**) in a similar manner as described above. The amino-protecting group of **11** was cleaved by treatment with concentrated hydrochloric acid to afford **8**.

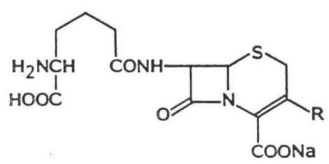
In routes A and B, the starting material, 7-ACA (**4**), was derived from cephalosporin C sodium salt (CCNa, **12**, Fig. 4). In order to find a more efficient synthetic route to FK027, we studied a new pathway (route C) by using deacetylcephalosporin C sodium salt (DCCNa, **13**, Fig. 4) as a starting material. Route C summarizes a new method for the preparation of **8**. Reaction of **13**, first with benzoyl chloride, and then diphenyldiazomethane gave the protected deacetylcephalosporin C (**14**). The hydroxymethyl group of **14** was converted to a chloromethyl group by treatment with PCl_5.

Scheme 1. Synthetic routes to **8**.



Reaction of the chloromethyl derivative (**15**) with PPh_3 and sodium iodide in DMF yielded the phosphonium salt, which by WITTIG reaction with formaldehyde in methylene chloride gave the 3-vinyl derivative (**16**). Cleavage of the 7-acyl side chain of **16** by the imino-chloride method afforded **8** in good yield.

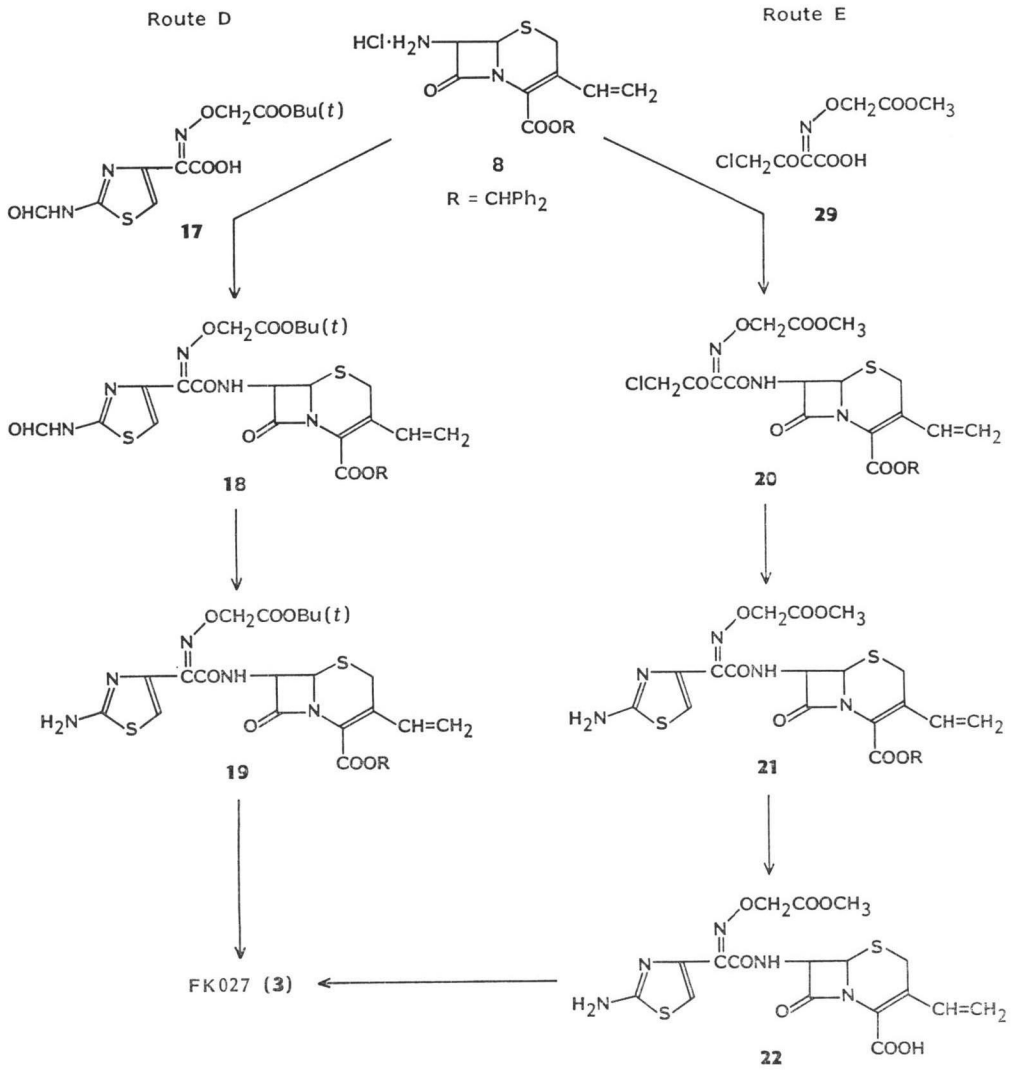
Fig. 4.



R=CH_2OCOCH_3 (CCNa, cephalosporin C sodium salt, **12**)
R=CH_2OH (DCCNa, deacetylcephalosporin C sodium salt, **13**)

Two synthetic routes to FK027 from **8** are summarized in Scheme 2. In route D, (*Z*)-2-(2-formamido-4-thiazolyl)-2-(*tert*-butoxycarbonylmethoxyimino)acetic acid (**17**)[18] was activated with Vilsmeier reagent prepared from DMF and phosphoryl chloride ($POCl_3$). The activated acid obtained above was condensed with **8** to afford the protected intermediate (**18**). Deprotection of the *N*-formyl group of **18** proceeded smoothly at room temperature in a methanolic solution containing concentrated hydrochloric acid to give the deformyl

Scheme 2. Synthetic routes to FK027 from **8**.

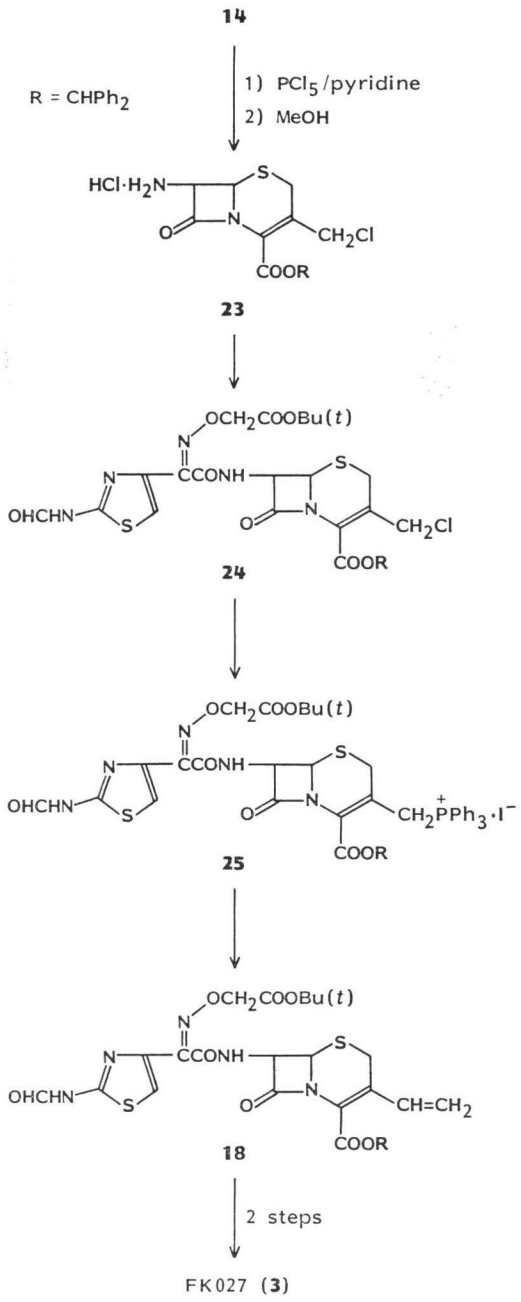


cephem (**19**). Removal of both *tert*-butyl and diphenylmethyl groups of **19** was carried out simultaneously by treating with trifluoroacetic acid (TFA) add anisole to afford 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic acid (**3**, FK027).

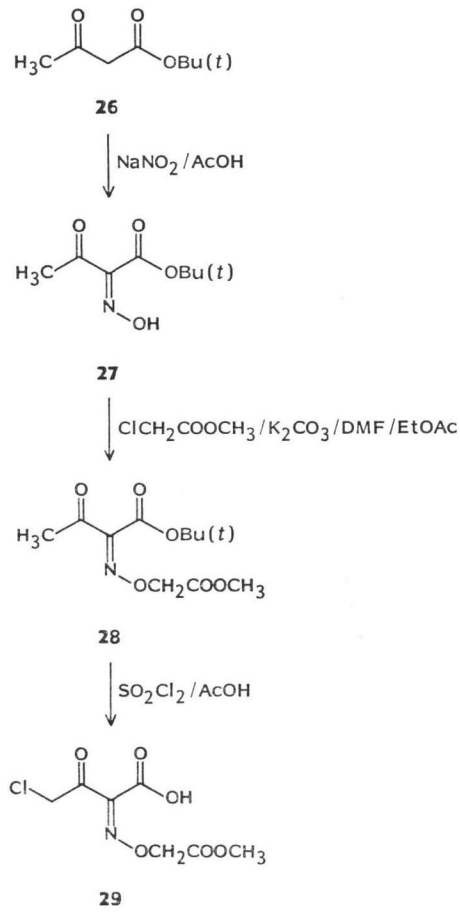
Route E outlines a novel synthetic pathway for the preparation of FK027. **8** was acylated with 4-chloro-2-methoxycarbonylmethoxyimino-3-oxobutyric acid (**29**), and then the reaction of the acylated cephem (**20**) with thiourea afforded the 2-aminothiazol cephem (**21**). The diphenylmethyl ester of **21** was cleaved by treatment with TFA and anisole to give the monoester (**22**), and finally hydrolysis of the methyl ester in **22** with sodium bicarbonate afforded FK027 (**3**).

Scheme 3 shows an alternative synthetic route to FK027 related to routes C and D. In this pathway, chlorination of the hydroxymethyl group and cleavage of the 7-acyl group in **14** were carried out in a one-pot process with PCl_5 and pyridine to give the 7-amino-3-chloromethyl derivative (**23**). The coupling reaction of **23** and the acid (**17**) was carried out by using Vilsmeier reagent as mentioned

Scheme 3. Synthetic route to FK027 from **14**.



Scheme 4. Synthetic route to **29**.



above. Subsequent reaction of the 3-chloromethyl cephem (**24**) with sodium iodide and PPh_3 afforded the corresponding phosphonium salt (**25**). **25** was submitted to WITTIG reaction with formaldehyde to give the vinyl derivative (**18**) already prepared by route D.

The synthesis of the novel acid (**29**) is outlined in Scheme 4. A diester (**28**) was prepared from *tert*-butyl acetoacetate (**26**) by treatment with sodium nitrite in acetic acid followed by alkylation with methyl chloroacetate. Chlorination of **28** with sulfuryl chloride in acetic acid and simultaneous cleavage of the *tert*-butyl ester furnished the acid (**29**).

Biological Results and Discussion

MIC values of FK027 against several Gram-positive and Gram-negative bacteria are shown in Table 1. For comparison, the MIC values of cephalexin (CEX), cefaclor (CCL) and amoxicillin

Table 1. Antibacterial activity of FK027 and reference drugs.

Organism	MIC (μg/ml)			
	FK027	CCL	CEX	AMPC
Staphylococcus aureus 209P JC-1	25	0.78	1.56	0.10
S. aureus Newman	12.5	0.78	3.13	0.20
S. epidermidis 68	3.13	0.39	1.56	0.20
Streptococcus pyogenes S-23*	0.10	0.20	0.78	≦0.025
S. pneumoniae III*	0.20	0.20	1.56	≦0.025
Haemophilus influenzae 1*	0.05	1.56	25	0.39
Neisseria gonorrhoeae PCL-783	≦0.025	0.10	0.78	0.10
Salmonella typhi T-287	≦0.025	0.39	6.25	0.78
Escherichia coli NIHJ JC-1	0.20	1.56	6.25	6.25
E. coli 28	0.39	1.56	6.25	6.25
Klebsiella pneumoniae NCTC 418	≦0.025	3.13	12.5	25
Proteus mirabilis 1	≦0.025	0.78	12.5	1.56
P. vulgaris IAM-1025	≦0.025	50	25	50
Providencia rettgeri 14	≦0.025	>100	25	100
Morganella morganii 55	1.56	>100	>100	>100
Citrobacter freundii 148	6.25	25	100	>100
Enterobacter cloacae 60	6.25	>100	>100	>100
E. aerogenes 20	12.5	100	>100	>100
Serratia marcescens 35	12.5	>100	>100	>100
Pseudomonas aeruginosa IAM-1095	25	>100	>100	>100
P. cepacia ATCC 25416	6.25	>100	>100	>100

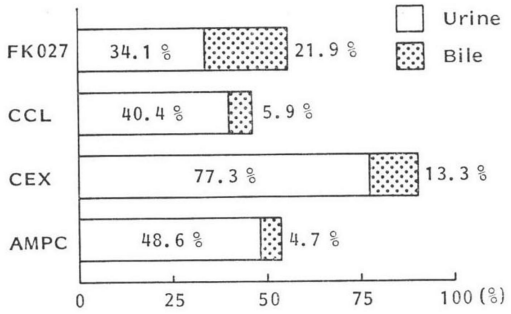
MH agar, streak method, 37°C, 18 hours, 10^6 cfu/ml.
* Supplement with 5% horse blood.

(AMPC) are listed as reference drugs in Table 1. FK027 was less active against *Staphylococcus aureus* than the reference drugs. However, FK027 showed high antibacterial activity similar to CCL and AMPC against Gram-positive bacteria such as *Streptococcus pyogenes* S-23 and *S. pneumoniae* III.

On the other hand, FK027 displayed much better activity against Gram-negative bacteria than the reference drugs. FK027 showed high inhibitory potency against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *P. vulgaris*, *Providencia rettgeri*, *Salmonella typhi*, *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Against most of these bacteria, except *N. gonorrhoeae*, CCL, CEX and AMPC were considerably less active than FK027. In particular, FK027 preserved excellent activity against *E. coli* 28 which is a cephalosporinase producer. FK027 was observed to be very stable to both penicillinase- and cephalosporinase-type β-lactamases.[10] In addition, FK027 showed considerable activity against opportunistic pathogens such as *Morganella morganii*, *Citrobacter freundii*, *Enterobacter cloacae*, *E. aerogenes*, *Serratia marcescens* and *Pseudomonas cepacia*. Against these strains, CCL, CEX and AMPC did not show useful activity. These results indicate that FK027 is a new oral cephalosporin possessing a widely expanded antibacterial activity comparable to those of the parenteral cephem antibiotics such as ceftizoxime.

The urinary and biliary excretion of FK027, CCL, CEX and AMPC in rats were followed for 24 hours after an oral dose of 100 mg/kg. As illustrated in Fig. 5, FK027 was excreted both in the urine (34.1%) and in the bile (21.9%). The urinary excretion of FK027 was significantly lower than that of CEX and AMPC but similar to that of CCL. In contrast, the biliary excretion of FK027 was higher than that of the three reference drugs. FK027 was excreted unchanged in the urine and bile of rats.

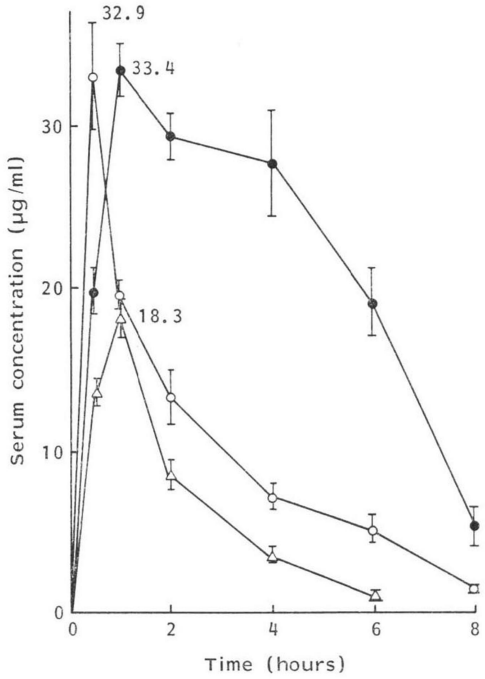
Fig. 5. Urinary and biliary excretion of FK027 and reference drugs.



Rat: SD strain, male, 6 weeks, (n=10).
Dose & route: 100 mg/kg, po.

Fig. 6. Serum concentration time curves of FK027 and reference drugs in rats after oral dose (100 mg/kg).

Rat: SD strain, male, 6 weeks, (n=10).
●: FK027, ○: cephalexin, △: amoxicillin.



Serum concentration time curves after oral administration (100 mg/kg) of FK027, CEX and AMPC to rats are shown in Fig. 6. The mean serum concentrations of FK027 in rats peaked at 33.4 μg/ml, 1 hour after oral dosing. The peak serum concentration of FK027 was similar to that of CEX but was higher than that of AMPC. The biological half-life of FK027 was 2.3 hours in rats. The biological half-life and the area under the serum concentration curve (AUC) of FK027 were longer and higher than those of the reference drugs.

As mentioned above, FK027 has some excellent biological properties such as the potent antibacterial activity against a wide range of Gram-negative bacteria including opportunistic pathogens, the high stability to β-lactamases[19] and the unique pharmacological properties of oral absorption and long-acting efficacy.[20] FK027 also had far greater protective activity than did reference drugs in mice infected with stock strains of Gram-negative bacteria.[21] FK027 completely differs in structure from the commercially available oral cephalosporins (Fig. 1), and its biological properties are what we desired for a new orally active cephalosporin. Thus we selected FK027 as a candidate for human trials.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and uncorrected. ^{1}H NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a Jeol-MH 100 NMR spectrometer using TMS as an internal standard. IR spectra were taken on a Hitachi 260-10 Spectrophotometer or Shimadzu IR-420 Spectrophotometer.

Column chromatography was carried out on macroporous non-ionic adsorption resin Diaion HP-20 (trademark, manufactured by Mitsubishi Chemical Industries Ltd.) or Merck silica gel 60 (70~230 mesh ASTM). Organic solvents were dried over anhydrous $MgSO_4$.

MIC's were determined by the known agar-dilution method using heart infusion agar (Difco) after incubation at 37°C for 18 hours and with an inoculum size of about 10^6 cfu/ml. *E. coli* 28 is a cephalosporin-resistant strain.

Sprague Drawley rats ($n=10$) were fasted overnight and orally dosed with 100 mg/kg of the test drugs. Urinary samples were collected for 24 hours after dosing. For bile collection another group of rats ($n=10$) was canulated with polyethylene tube into the bile duct and the test drugs were given orally at doses of 100 mg/kg.

FK027 concentrations in the urine, bile and serum were measured by the disc-plate diffusion method using *E. coli* ATCC 39188 as the test organism and nutrient agar (Difco) as the test medium. The reference drugs were assayed in the same way using *Bacillus subtilis* ATCC 6633 as the test organism and sodium citrate agar as the test medium.

Diphenylmethyl 7β-Phenylacetamido-3-vinyl-3-cephem-4-carboxylate (**7**)

To a soln of **5** (64.5 g, 125 mmol) in THF (250 ml) was dropwise adddd phosphorous tribromide (12.5 g, 46.4 mmol) at $-5\sim0$°C with stirring. After being stirred at this temp for 20 minutes, the reaction mixture was poured into H_2O (370 ml), and extracted with EtOAc (250 ml). The separated organic layer was washed with brine, dried and evaporated *in vacuo*. The residue (**6**) was dissolved in EtOAc (250 ml), and triphenylphosphine (PPh_3) (39.5 g, 150 mmol) was added. After stirring at room temp for 5 hours, the precipitated phosphonium salt was collected by filtration, washed with EtOAc, and dried to give 84.0 g (80%) of the phosphonium salt. To a soln of the phosphonium salt (84 g, 100 mmol) in methylene chloride (CH_2Cl_2) (600 ml) were added 36% aq formaldehyde (417 ml, 5.0 mol) and a soln of sodium carbonate (Na_2CO_3) (53 g, 0.5 mol) in H_2O (200 ml) at room temp. After being stirred at this temp for 90 minutes, the reaction mixture was neutralized with 20% sulfuric acid. The separated organic layer was washed with brine, dried, and evaporated *in vacuo*. The residue was triturated with MeOH to afford 29 g (45.5%, based on **5**) of **7**: IR (Nujol) 3280, 1772, 1722, 1667 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.57, 4.00 (2H, ABq, $J=18$ Hz), 3.60 (2H, s), 5.23 (1H, d, $J=5$ Hz), 5.32 (1H, d, $J=11$ Hz), 5.65 (1H, d, $J=18$ Hz), 5.83 (1H, dd, $J=5$ Hz, 8 Hz), 6.83 (1H, dd, $J=11$ Hz, 18 Hz), 7.03 (1H, s), 7.2~7.8 (15H, m), 9.23 (1H, d, $J=8$ Hz).

Diphenylmethyl 7-Amino-3-vinyl-3-cephem-4-carboxylate Hydrochloride (**8**)

To a suspension of PCl_5 (156 g, 0.75 mol) in CH_2Cl_2 (1.5 liters) was added pyridine (60 g, 0.75 mol) under ice-cooling, and the suspension was stirred at this temp for 1 hour. Then, **7** was added to the suspension at 5°C. After being stirred at 8~10°C for 90 minutes, the reaction mixture was cooled to -35°C. To the cooled mixture was added MeOH (1.0 liter, 25 mol) all at once, and stirred under keeping the temp below -10°C for 75 minutes. To the resulting soln was added H_2O (200 ml) at -5°C. After removing the solvent *in vacuo*, the residue was triturated with H_2O (50 ml) and diethyl ether (Et_2O) (500 ml). The resulting ppt was collected by filtration, washed with H_2O (300 ml) and Et_2O (300 ml) to give 86.9 g (81.2%) of **8**: mp 172~173°C (dec); IR (Nujol) 3350, 1767, 1709 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.72, 4.13 (2H, ABq, $J=18$ Hz), 5.27 (1H, d, $J=5$ Hz), 5.40 (1H, d, $J=5$ Hz), 5.47 (1H, d, $J=11$ Hz), 5.83 (1H, d, $J=18$ Hz), 7.03 (1H, s), 7.07 (1H, dd, $J=11$ Hz, 18 Hz), 7.1~7.8 (10H, m).

Anal Calcd for $C_{22}H_{21}ClN_2O_3S$: C 61.60, H 4.93, N 6.53, S 7.47, Cl 8.27.
Found: C 61.57, H 4.93, N 6.41, S 7.30, Cl 8.55.

Diphenylmethyl 7β-(*o*-Hydroxy)benzylidenamino-3-hydroxymethyl-3-cephem-4-carboxylate (**9**) from 7-ACA (**4**)

To a suspension of **4** (18.7 g, 67 mmol) in a mixture of H_2O (120 ml) and MeOH (120 ml) was added dropwise a soln of sodium hydroxide (5.5 g, 142 mmol) in H_2O (18 ml) at -20°C. The mixture was stirred at $-20\sim-10$°C for 25 minutes and neutralized to pH 7.5 with concd hydrochloric acid (HCl). To the mixture was added salicylaldehyde (10.7 g, 87.6 mmol) at 15°C. Stirring was continued at this temp for 1 hour, then to the mixture was added a soln of diphenyldiazomethane (15.7 g, 80.9 mmol) in EtOAc (85 ml). The mixture was stirred for 1 hour. During the period of the reaction, the mixture was kept at pH 4.0~4.5 with 1 N HCl. The resulting mixture was extracted with EtOAc (550 ml). The separated organic layer was washed with brine, dried and concd under reduced pressure. The residue was triturated with petroleum ether to give 29.6 g (78.1%) of **9**: mp 97~98.5°C (dec); IR (Nujol) 3470, 1760, 1700, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.70 (2H, s), 4.30 (2H, s), 5.15

(1H, br s), 5.45 (1H, d, J=5 Hz), 5.71 (1H, d, J=5 Hz), 6.96 (1H, s), 7.0~7.7 (14H, m), 8.80 (1H, s).

Anal Calcd for $C_{25}H_{24}N_2O_5S \cdot 0.5H_2O$: C 66.00, H 4.94, N 5.50.
Found: C 66.41, H 4.66, N 5.50.

Diphenylmethyl 7β-(*o*-Hydroxy)benzylidenamino-3-chloromethyl-3-cephem-4-carboxylate (**10**)

To a soln of **9** (1.01 g, 2 mmol) in CH_2Cl_2 (10 ml) was added PCl_5 (0.46 g, 2.2 mmol) at −30°C followed by the addition of pyridine (0.176 g, 2.2 mmol). The mixture was stirred at −30~−20°C for 1 hour and poured into ice-water. The separated organic layer was washed with brine, dried and evaporated *in vacuo* to give 0.9 g (85.7%) of **10**: mp 180.5~182.0°C (dec); IR (Nujol) 1775, 1710, 1605, 1500 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.67, 4.08 (2H, ABq, J=18 Hz), 4.55 (2H, s), 5.40 (1H, s), 5.50 (1H, d, J=5 Hz), 5.85 (1H, d, J=5 Hz), 7.05 (1H, s), 7.2~7.8 (14H, m), 8.95 (1H, s).

Diphenylmethyl 7β-(*o*-Hydroxy)benzylidenamino-3-vinyl-3-cephem-4-carboxylate (**11**) from **10**

To a cooled soln of **10** (4.0 g, 7.6 mmol) in DMF (10 ml) were added PPh_3 (2.2 g, 8.4 mmol) and sodium iodide (1.37 g, 9.1 mmol) with stirring. After stirring at room temp for 2 hours, the reaction mixture was poured into isopropyl alcohol (iPA) (250 ml) and the resulting ppt was collected by filtration. To a soln of the above ppt (6.7 g) in a mixture of CH_2Cl_2 (10 ml) and H_2O (5 ml) was added 36% aq formaldehyde (17.4 ml). Then the reaction mixture was adjusted to pH 9.0 with 10% aq Na_2CO_3. After stirring at room temp for 1 hour, the mixture was extracted with CH_2Cl_2 (20 ml). The separated organic layer was washed with brine, dried and concd under reduced pressure to give 3.0 g (78.9%) of **11**: mp 180.5~182°C (dec); IR (Nujol) 1770, 1710, 1620, 1580 cm^{-1}; ^1N NMR (DMSO-d_6) δ 3.67, 4.08 (2H, ABq, J=18 Hz), 5.30 (1H, d, J=11 Hz), 5.40 (1H, d, J=5 Hz), 5.65 (1H, d, J=18 Hz), 5.75 (1H, d, J=5 Hz), 6.90 (1H, dd, J=11 Hz, 18 Hz), 7.05 (1H, s), 7.2~7.9 (14H, m), 8.95 (1H, s).

Anal Calcd for $C_{29}H_{24}N_2O_4S$: C 70.14, H 4.87, N 5.64.
Found: C 70.53, H 4.78, N 5.65.

Preparation of **8** from **11**

To a suspension of **11** (18.96 g, 38.2 mmol) in a mixture of EtOAc (135 ml) and EtOH (34 ml) was added concd HCl (5.7 ml) at room temp. The mixture was stirred for 1.5 hours and the resulting ppt was collected by filtration, washed with EtOAc and dried to give 11.32 g (69.1%) of **8**.

Diphenylmethyl 7β-[5-Benzamido-5-(diphenylmethoxycarbonyl)pentanamido]-3-hydroxymethyl-3-cephem-4-carboxylate (**14**)

To a soln of deacetylcephalosporin C sodium salt (DCCNa) (**13**) (118.6 g, 0.3 mol) in H_2O (1.0 liter) and Me_2CO (0.6 liter) was added benzoyl chloride (42.1 g, 0.3 mol) at 10~15°C. During the period of addition, the reaction mixture was kept at pH 6.5~7.5 with 20% aq Na_2CO_3. After the addition, the reaction mixture was stirred at this temp for 1 hour. After removal of the Me_2CO under reduced pressure, the aq soln was washed with EtOAc. To the separated aq soln was added EtOAc (300 ml), and to the mixture was added diphenyldiazomethane (135.8 g, 0.75 mol) in EtOAc (1.0 liter). The mixture was adjusted to pH 3.5 with concd HCl, and stirred at room temp for 1.5 hours. The resulting mixture was adjusted to pH 2.5 with concd HCl. The separated organic layer was washed with brine, dried and evaporated *in vacuo*. The residue was dissolved in Me_2CO (400 ml), and pulverized with diisopropyl ether (iPE) (4.0 liters) to give 224.8 g (92.5%) of **14**: IR (Nujol) 3280, 1765, 1733, 1657, 1638 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.5~2.5 (6H, m), 3.38 (2H, s), 3.65 (2H, s), 4.27 (2H, m), 4.67 (1H, m), 5.15 (1H, d, J=5 Hz), 5.77 (1H, dd, J=5 Hz, 8 Hz), 6.87 (1H, s), 6.95 (1H, s), 7.1~7.8 (25H, m), 8.86 (2H, m).

Diphenylmethyl 7β-[5-Benzamido-5-(diphenylmethoxycarbonyl)pentanamido)-3-chloromethyl-3-cephem-4-carboxylate (**15**)

To a soln of **14** (100 g, 0.123 mol) in CH_2Cl_2 (600 ml) was added PCl_5 (25.6 g, 0.123 mol) at −30°C. The mixture was stirred at this temp for 30 minutes. Then, to the mixture was added pyridine (9.8 g, 0.123 mol) at the same temp. After being stirred at −20~−10°C for 1 hour, the mixture was poured into a mixture of CH_2Cl_2 (500 ml) and H_2O (300 ml). The separated CH_2Cl_2 layer was washed with

brine, dried, and evaporated *in vacuo*. The residue was triturated with iPE to give 114.5 g (98%) of **15** as a powder: IR (Nujol) 3250, 1784, 1725, 1644 (br) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.5~2.5 (6H, m), 3.60 (2H, br s), 3.45 (2H, br s), 4.6 (1H, m), 5.21 (1H, d, J=5 Hz), 5.80 (1H, dd, J=5 Hz, 8 Hz), 6.83 (1H, s), 7.0 (1H, s), 7.2~8.1 (25H, m), 8.85 (1H, d, J=7 Hz), 8.93 (1H, d, J=8 Hz).

Diphenylmethyl 7β-[5-Benzamido-5-(diphenylmethoxycarbonyl)pentanamido]-3-vinyl-3-cephem-4-carboxylate (**16**)

To a soln of **15** (102 g, 0.123 mol) in DMF (300 ml) was added sodium iodide (18.4 g, 0.123 mol) at 35°C. The mixture was stirred at this temp for 30 minutes. To the mixture was added PPh_3 (48.5 g, 0.185 mol), and the mixture was stirred at 35~38°C for 1 hour. The resulting soln was concd under reduced pressure to two-third of its original volume. The resulting soln was added dropwise to iPA (5.0 liters) and the ppt was collected by filtration, washed with iPE, and dried under reduced pressure to give 123.5 g (85.0%) of phosphonium salt. To a soln of the phosphonium salt (123.5 g, 0.104 mol) in CH_2Cl_2 (1.0 liter) was added 36% aq formaldehyde (300 ml) at 25°C. The mixture was kept on adjusting to pH 9.0 with 20% Na_2CO_3 and stirred at 25°C for 2 hours. The reaction mixture was adjusted to pH 5.0 with 10% HCl, and the separated organic layer was washed with brine, and evaporated *in vacuo*. The residue was triturated with EtOAc to give 63.5 g (75.8%) of **16** as a powder; IR (Nujol) 3290, 1770, 1728, 1714, 1652 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.5~2.6 (6H, m), 3.51, 3.88 (2H, ABq, J=16 Hz), 4.62 (1H, m), 5.21 (1H, d, J=5 Hz), 5.26 (1H, d, J=11 Hz), 5.60 (1H, d, J=18 Hz), 5.78 (1H, dd, J=5 Hz, 8 Hz), 6.83 (1H, dd, J=11 Hz, 18 Hz), 6.86 (1H, s), 7.00 (1H, s), 7.2~8.1 (25H, m), 8.86 (1H, d, J=7 Hz), 8.97 (1H, d, J=8 Hz).

Diphenylmethyl 7-Amino-3-vinyl-3-cephem-4-carboxylate Hydrochloride (**8**) from **16**

To a suspension of PCl_5 (15.5 g, 74.4 mmol) in CH_2Cl_2 (200 ml) was added pyridine (5.9 g, 74.4 mmol) at 5°C, and the mixture was stirred at this temp for 20 minutes. To the suspension was added **16** at 5°C. After being stirred at the same temp for 2 hours, the mixture was cooled to −40°C. To the soln was added MeOH (120 ml) that was previously cooled to −40°C all at once. The temp of the mixture rose slowly to 20°C over 1 hour. The resulting soln was concd under reduced pressure, and triturated with a mixture of EtOAc (300 ml) and H_2O (50 ml). The ppt was collected by filtration, washed twice with iPA (50 ml), and then with iPE (50 ml) to give 8.4 g (79.0%) of **8** as crystals.

Diphenylmethyl 7β-[(*Z*)-2-(2-Formamido-4-thiazolyl)-2-(*tert*-butoxycarbonylmethoxyimino)-acetamido]-3-vinyl-3-cephem-4-carboxylate (**18**)

To a mixture of DMF (3.66 g, 50.1 mmol) and THF (80 ml) was dropwise added $POCl_3$ (7.7 g, 50.1 mmol) at −5~0°C under stirring, and the mixture was stirred at this temp for 30 minutes to prepare Vilsmeier reagent. To the above mixture was added the acid (**17**) (13.8 g, 41.8 mmol) under ice-cooling, and the reaction mixture was stirred at the same temp for 1 hour to prepare an activated soln of **17**. To a soln of **8** (15 g, 34.9 mmol) and *N*-(trimethylsilyl)acetamide (MSA) (32 g, 244 mmol) in EtOAc (150 ml) was added the above activated acid soln at −20°C, and the mixture was stirred at this temp for 30 minutes. To the reaction mixture were added EtOAc and H_2O. The separated organic layer was washed with 5% aq $NaHCO_3$ and brine, and dried. The solvent was evaporated *in vacuo*, and the residue was triturated with iPE to afford 23.1 g (97.5%) of **18**; IR (Nujol) 3250, 1780, 1720, 1680, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.45 (9H, s), 3.50, 3.95 (2H, ABq, J=18 Hz), 4.64 (2H, s), 5.32 (1H, d, J=5 Hz), 5.32 (1H, d, J=11 Hz), 5.65 (1H, d, J=18 Hz), 5.97 (1H, dd, J=5 Hz, 8 Hz), 6.82 (1H, dd, J=11 Hz, 18 Hz), 7.00 (1H, s), 7.2~7.7 (11H, m), 8.57 (1H, s), 9.7 (1H, d, J=8 Hz), 12.73 (1H, br s).

Diphenylmethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(*tert*-butoxycarbonylmethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylate (**19**)

To a mixture of the *N*-formyl derivative (**18**) (19.0 g, 28.0 mmol) in MeOH (380 ml) was added concd HCl (11.6 g, 112 mmol) at room temp, and the mixture was stirred at the same temp for 1 hour. The resultant mixture was neutralized with 5% aq $NaHCO_3$ and concd under reduced pressure. The residue was dissolved in EtOAc, and the organic layer was washed with brine and dried. The solvent was evaporated *in vacuo*, and the residue was triturated with iPE to afford 15.3 g (84.1%) of **19**: IR

(Nujol) 3440, 3260, 3100, 1780, 1720, 1660, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.44 (9H, s), 3.58, 4.03 (2H, ABq, J=18 Hz), 4.58 (2H, s), 5.29 (1H, d, J=5 Hz), 5.30 (1H, d, J=11 Hz), 5.63 (1H, d, J=18 Hz), 5.90 (1H, dd, J=5 Hz, 8 Hz), 6.83 (1H, s), 6.85 (1H, dd, J=11 Hz, 18 Hz), 6.93 (1H, s), 7.2~7.8 (10H, m), 9.56 (1H, d, J=8 Hz).

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic Acid (3, FK027)

To a mixture of the ester (**19**) (15.0 g, 23 mmol) and anisole (15 ml) was added TFA (60 ml) under ice-cooling, and the mixture was stirred at room temp for 80 minutes. The reaction mixture was dropwise added to iPE (600 ml) under stirring to form a ppt. The collected ppt was dissolved in 5% aq $NaHCO_3$, and the aq soln was washed with EtOAc. Then, the aq soln was adjusted to pH 6.0 with 5% HCl, and was subjected to column chromatography on macroporous non-ionic adsorption resin Diaion HP-20. The desired product was eluted with H_2O, and the eluate was acidified to pH 2.3 with 10% HCl under ice-cooling. The resulting ppt was collected by filtration and dried to afford 3.55 g (34.1%) of FK027 (**3**): IR (Nujol) 3350, 1770, 1733, 1668, 1590, 1540, 1095 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.42, 3.87 (2H, q, J=18 Hz), 4.60 (2H, s), 5.18 (1H, d, J=5 Hz), 5.29 (1H, d, J=11.5 Hz), 5.57 (1H, d, J=18 Hz), 5.79 (1H, dd, J=5 Hz, 8 Hz), 6.81 (1H, s), 6.91 (1H, dd, J=11.5 Hz, 18 Hz), 7.23 (2H, br s), 9.52 (1H, d, J=8 Hz).

Anal Calcd for $C_{16}H_{15}N_5O_7S_2 \cdot 3H_2O$: C 37.87, H 4.17, N 13.80, S 12.63.
Found: C 37.95, H 4.05, N 13.73, S 12.38.

Diphenylmethyl 7β-(4-Chloro-2-methoxycarbonylmethoxyimino-3-oxobutyramido)-3-vinyl-3-cephem-4-carboxylate (**20**)

To a soln of Vilsmeier reagent prepared from $POCl_3$ (7.1 g, 46.3 mmol) and DMF (3.4 g, 46.3 mmol) in THF (30 ml) was added **29** (10.0 g, 42.1 mmol) at 5°C. After being stirred at the same temp for 1 hour, the activated acid soln was added to a mixture of **8** (16.3 g, 38.0 mmol) and MSA (40 g, 305 mmol) in EtOAc (160 ml) at −30°C all at once. The mixture was stirred at −15~−10°C for 1 hour and poured into ice-water (150 ml). The separated organic layer was washed with 5% aq $NaHCO_3$ and brine. After being dried, the soln was concd under reduced pressure. The resulting residue was triturated with *n*-hexane to give 21.8 g (93.7%) of **20** as crystals: mp 171~173°C (dec); IR (Nujol) 3260, 1770, 1750, 1708, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.55, 3.94 (2H, ABq, J=18 Hz), 3.65 (3H, s), 4.75 (2H, s), 4.87 (2H, s), 5.22 (1H, d, J=5 Hz), 5.23 (1H, d, J=11 Hz), 5.57 (1H, d, J=17 Hz), 5.85 (1H, dd, J=5 Hz, 8 Hz), 6.71 (1H, dd, J=11 Hz, 17 Hz), 6.90 (1H, s), 7.28 (10H, m), 9.46 (1H, d, J=8 Hz).

Anal Calcd for $C_{29}H_{26}ClN_3O_8S \cdot H_2O$: C 55.30, H 4.48, N 6.67, S 5.09, Cl 5.63.
Found: C 55.57, H 4.60, N 6.72, S 5.29, Cl 5.38.

Diphenylmethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(methoxycarbonylmethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylate (**21**)

To a mixture of **20** (2.0 g, 3.27 mmol), THF (10 ml) and H_2O (10 ml) were added thiourea (0.5 g, 6.57 mmol) and sodium acetate (1.34 g, 16.4 mmol). After being stirred at 40°C for 4 hours, the mixture was extracted with EtOAc (50 ml). The separated organic layer was washed twice with brine, dried, and evaporated *in vacuo* to give 2.0 g (96%) of **21**: mp 182~184°C (dec); IR (Nujol) 3440, 3260, 1778, 1740, 1720, 1662, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.3~3.9 (2H, m), 3.63 (3H, s), 4.67 (2H, s), 5.23 (1H, d, J=5 Hz), 5.26 (1H, d, J=11 Hz), 5.60 (1H, d, J=18 Hz), 5.86 (1H, dd, J=5 Hz, 8 Hz), 6.75 (1H, dd, J=11 Hz, 18 Hz), 6.76 (1H, s), 6.88 (1H, s), 7.3 (10H, m), 9.56 (1H, d, J=8 Hz).

Anal Calcd for $C_{30}H_{27}N_5O_7S_2$: C 56.86, H 4.29, N 11.05, S 10.12.
Found: C 57.12, H 4.17, N 11.07, S 10.45.

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(methoxycarbonylmethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic Acid (**22**)

To a soln of **21** (2.0 g, 3.2 mmol) in CH_2Cl_2 (4 ml) were added anisole (2 ml) and TFA (6 ml) under ice-cooling. After stirring 30 minutes at the same temp, the mixture was poured into iPE (30 ml). The resulting ppt was collected by filtration, washed with iPE, and dissolved in a mixture of EtOAc

(30 ml) and 5% aq $NaHCO_3$ (20 ml). After being adjusted to pH 2.3 with 5% HCl, the organic layer was separated, washed with brine, and dried. The solvent was evaporated *in vacuo* to give 1.22 g (82%) of **22**: mp 180~185°C (dec); IR (Nujol) 3240, 1760, 1724, 1650 (br) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.50, 3.88 (2H, ABq, J=18 Hz), 3.65 (3H, s), 4.67 (2H, s), 5.17 (1H, d, J=5 Hz), 5.28 (1H, d, J=11 Hz), 5.51 (1H, d, J=18 Hz), 5.75 (1H, dd, J=5 Hz, 8 Hz), 6.73 (1H, s), 6.88 (1H, dd, J=11 Hz, 18 Hz), 9.50 (1H, d, J=8 Hz).

Anal Calcd for $C_{17}H_{17}N_5O_7S_2 \cdot 1.5H_2O$: C 41.30, H 4.08, N 14.16.
Found: C 41.40, H 3.72, N 14.26.

Preparation of FK027 (**3**) from **22**

A soln of **22** (2.0 g, 4.3 mmol) and $NaHCO_3$ (1.8 g, 21.4 mmol) in H_2O (40 ml) was stirred at 40°C for 7 hours. The resulting soln was adjusted to pH 6.0 with 10% HCl and subjected to column chromatography on Diaion HP-20 (20 ml). The column was eluted with H_2O, and the fraction containing the desired compound was acidified to pH 2.1 with 10% HCl under ice-cooling. After stirring at the same temp for 1 hour, the resulting ppt was collected by filtration, washed with H_2O and dried to afford 0.9 g (41.0%) of FK027 (**3**).

Diphenylmethyl 7-Amino-3-chloromethyl-3-cephem-4-carboxylate Hydrochloride (**23**)

To a suspension of PCl_5 (216 g, 1.04 mol) in CH_2Cl_2 was added pyridine (82 g, 1.04 mol) under ice-cooling. The mixture was stirred at the same temp for 15 minutes, and cooled to −40°C. To the cooled suspension was added **14** (210 g, 0.26 mol), and the reaction mixture was stirred at −35~−30°C for 3 hours. To the mixture was dropwise added MeOH (1.0 liter). During the period of the addition, the reaction temp was maintained below −20°C. After the addition, the mixture was stirred at −10°C for 30 minutes. The resulting mixture was concd under reduced pressure, and the residue was dissolved in CH_2Cl_2 (1.0 liter). To the CH_2Cl_2 soln was added H_2O (500 ml) with stirring followed by addition of iPE (1.2 liters). After stirring under ice-cooling for 30 minutes, the ppt was collected by filtration. The crystalline ppt was at first washed with iPA and next with iPE to give 70.0 g (59.6%) of **23**: IR (Nujol) 1775, 1710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.76 (2H, br s), 4.50 (2H, br s), 5.15~5.40 (2H, m), 6.95 (1H, s), 7.4 (10H, m).

Diphenylmethyl 7β-[(*Z*)-2-(2-Formamido-4-thiazolyl)-2-(*tert*-butoxycarbonylmethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate (**24**)

To a suspension of Vilsmeier reagent prepared from $POCl_3$ (14.8 g, 96.5 mmol) and DMF (7.07 g, 96.5 mmol) in EtOAc (250 ml) was added **17** (29.0 g, 88.0 mmol) under ice-cooling. The mixture was stirred at the same temp for 30 minutes to prepare an activated acid soln. To a soln of **23** (36.1 g, 80.0 mmol) and MSA (63 g, 480 mmol) in EtOAc (400 ml) was added the activated acid soln at −15°C. After being stirred at −20~0°C for 1 hour, the mixture was poured into ice-water (500 ml). The separated organic layer was washed with 5% aq $NaHCO_3$, then with brine, and dried. The organic soln was concd under reduced pressure. The residue was pulverized with Et_2O to give 49.7 g (85.0%) of **24** as a powder: IR (Nujol) 3200, 1780, 1720, 1680, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.42 (9H, s), 3.48, 3.89 (2H, ABq, J=18 Hz), 4.43 (2H, s), 4.64 (2H, s), 5.27 (1H, d, J=5 Hz), 5.98 (1H, dd, J=5 Hz, 8 Hz), 6.96 (1H, s), 7.0~7.6 (11H, m), 8.50 (1H, s), 9.64 (1H, d, J=8 Hz), 12.58 (1H, br s).

[4-Diphenylmethoxycarbonyl-7β-(2-(*tert*-butoxycarbonylmethoxyimino)-2-(2-formamido-4-thiazolyl))acetamido-3-cephem-3-ylmethyl]triphenylphosphonium Iodide (**25**)

To a soln of **24** (7.6 g, 10.5 mmol) in Me_2CO (70 ml) was added sodium iodide (4.5 g, 22.7 mmol), and the mixture was stirred at room temp for 2.5 hours. The resulting mixture was poured into a mixture of EtOAc (200 ml) and brine (100 ml). The separated organic layer was washed with 10% aq sodium thiosulfate and brine. After being dried, the organic soln was evaporated *in vacuo*. The residue and PPh_3 (5.2 g, 19.8 mmol) was dissolved in EtOAc (100 ml), and the mixture was stirred at room temp for 1 hour. The resulting ppt was collected by filtration, washed with EtOAc and dried under reduced pressure to give 6.5 g (62.6%) of **25**: IR (Nujol) 1785, 1710, 1680, 1530 cm^{-1}.

Diphenylmethyl 7β-[(*Z*)-2-(2-Formamido-4-thiazolyl)-2-(*tert*-butoxycarbonylmethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylate (**18**) from **25**

A mixture of **25** (0.59 g, 0.6 mmol), CH_2Cl_2 (20 ml), H_2O (2 ml) and 36% aq formaldehyde (1.0 ml) was stirred at 30~35°C for 3 hours. During the period of the reaction, the mixture was kept on adjusting to pH 8.0 with 20% aq Na_2CO_3. The resulting mixture was adjusted to pH 2.0 with 10% HCl, and extracted with CH_2Cl_2. The extract was washed with brine, dried, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (5 g) using benzene - EtOAc, 2: 1, as an eluent to give 0.14 g (34.3%) of **18**.

Preparation of *tert*-Butyl 2-Methoxycarbonylmethoxyimino-3-oxo-butyrate (**28**) from *tert*-Butyl Acetoacetate (**26**)

To a soln of *tert*-butyl acetoacetate (500 g, 3.16 mol) in AcOH (500 ml) was added a soln of sodium nitrite (229 g, 3.32 mol) in H_2O (400 ml) under ice-cooling. During the addition, the reaction temp was maintained below 15°C. After the addition, the mixture was stirred at 15°C for 30 minutes. After removal of AcOH under reduced pressure, the residue was dissolved in EtOAc, and washed with 5% aq $NaHCO_3$. The separated organic layer was washed with brine, dried, and evaporated *in vacuo* to give 590 g (98%) of **27** as an oil. To a soln of **27** (590 g) in EtOAc (885 ml) and DMF (885 ml) were added methyl chloroacetate (342 g, 3.16 mol) and potassium carbonate (K_2CO_3) (436 g, 3.16 mol) at room temp with stirring. An additional amount of K_2CO_3 (218 g, 1.58 mol) was added 30 minutes later. After stirring at room temp for 15 hours, the mixture was poured into ice-water (2.0 liters) and extracted with EtOAc (1.0 liter). The separated EtOAc layer was washed with H_2O (500 ml) three times, dried, and concd under reduced pressure to give 776.8 g (94.9% based on **26**) of **28** as an oil: IR (film) 1760, 1740, 1690, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.40 (9H, s), 2.23 (3H, s), 3.62 (3H, s), 4.86 (2H, s).

Preparation of 4-Chloro-2-methoxycarbonylmethoxyimino-3-oxobutyric Acid (**29**) from **28**

To a soln of **28** (51.9 g, 0.2 mol) in AcOH (52 ml) was added sulfuryl chloride (121.5 g, 0.9 mol) at 58~60°C over a 3.5-hour period. After an additional hour, the reaction mixture was cooled and concd under reduced pressure. The residue was dissolved in EtOAc (200 ml) and the EtOAc soln was washed with brine three times. After being dried, the solvent was evaporated *in vacuo*. The resulting crystalline ppt was collected by filtration, and recrystallized from a mixture of *n*-hexane and iPE to afford 13.4 g (28.2%) of **29** as colorless needles: mp 134~135°C (dec); IR (Nujol) 1745, 1720, 1701, 1604 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.66 (3H, s), 4.79 (2H, s), 4.92 (2H, s), 10.7 (1H, br s).

Anal Calcd for $C_7H_8ClNO_6$: C 35.39, H 3.39, N 5.90, Cl 14.92.
Found: C 35.43, H 3.51, N 5.82, Cl 14.52.

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References

1) HEYMES, R.; A. LUTZ & E. SCHRINNER: Experimental evaluation of HR 756, a new cephalosporin derivative: Pre-clinical study. Infection 5: 259~260, 1977
2) OCHIAI, M.; O. AKI, A. MORIMOTO, T. OKADA & Y. MATSUSHITA: New cephalosporin derivatives with high antibacterial activities. Chem. Pharm. Bull. 25: 3115~3117, 1977
3) REINER, R.; U. WEISS, U. BROMBACHER, P. LANZ, M. MONTAVON, A. FURLENMEIER, P. ANGEHRN & P. J. PROBST: Ro 13-9904/001, a novel potent and long-acting parenteral cephalosporin. J. Antibiotics 33: 783~786, 1980
4) NAKAO, H.; H. YANAGISAWA, B. SHIMIZU, M. KANEKO, M. NAGANO & S. SUGAWARA: A new semisynthetic 7α-methoxycephalosporin, CS-1170: 7β-[[(Cyanomethyl)thio]acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid. J. Antibiotics 29: 554~558, 1976
5) IWANAMI, M.; T. MAEDA, M. FUJIMOTO, Y. NAGANO, N. NAGANO, A. YAMAZAKI, T. SHIBANUMA, K.

TAMAZAWA & K. YANO: Synthesis of new cephamycin derivatives and a novel rearrangement between isothiazolethioacetamides and 1,3-dithietanecarboxamides. Chem. Pharm. Bull. 28: 2629~2636, 1980

6) OKUDA, H.; W. NAGATA, M. YOSHIOKA, M. NARISADA, T. YOSHIDA, Y. HARADA & H. YAMADA: Discovery and development of moxalactam (6059-S): The chemistry and biology of 1-oxacephems. *In* Medicinal Research Reviews. *Ed.*, G. DESTEVENS, Vol. 1, pp. 217~248, John Wiley & Sons, New York, 1981
7) UEO, K.; Y. FUKUOKA, T. HAYASHI, T. YASUDA, H. TAKI, M. TAI, Y. WATANABE, I. SAIKAWA & S. MITSUHASHI: In vitro and in vivo antibacterial activity of T-1220, a new semisynthetic penicillin. Antimicrob. Agents Chemother. 12: 455~460, 1977
8) SCARTAZZINI, R. & H. BICKEL: New orally active cephalosporins. Heterocycles 7: 1165~1188, 1977
9) TAKASUGI, H.; Z. TOZUKA & T. TAKAYA: Studies on β-lactam antibiotics. VI. Effect on antibacterial activity of α-substituents in the 2-(2-amino-4-thiazolyl)acetyl side chain of a cephalosporin nucleus. J. Antibiotics 36: 36~41, 1983
10) TAKAYA, T.; T. KAMIMURA, H. KOJO, Y. MINE, S. NISHIDA, S. GOTO & S. KUWAHARA: Ceftizoxime (FK 749), a new parenteral cephalosporin: *In vitro* and *in vivo* antibacterial activities. *In* Current Chemotherapy and Infections Disease, *Ed.*, J. D. NELSON & C. GRASSI, Vol. 1, pp. 255. The American Society of Microbiology, Washington, DC, 1980
11) TAKAYA, T.; H. TAKASUGI, T. MASUGI, T. CHIBA, H. KOCHI, T. TAKANO & H. NAKANO: Structure-activity relationships of sodium 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylate(ceftizoxime) and its related compounds. Nippon Kagaku Kaishi 5: 785~804, 1981
12) NAKANO, H.: Structure-activity relationships related to ceftizoxime (FK 749). *In* Medicinal Research Reviews, *Ed.*, G. DESTEVENS, Vol. 1, pp. 127~157, John Wiley & Sons, New York, 1981
13) YAMANAKA, H.; H. TAKASUGI, T. MASUGI, H. KOCHI, K. MIYAI & T. TAKAYA: Studies on β-lactam antibiotics. VIII. Structure-activity relationships of 7β-[(*Z*)-2-carboxymethoxyimino-2-arylacetamido]-3-cephem-4-carboxylic acids. J. Antibiotics 38: 1068~1076, 1985
14) YAMANAKA, H.; K. KAWABATA, K. MIYAI, H. TAKASUGI, T. KAMIMURA, Y. MINE & T. TAKAYA: Studies on β-lactam antibiotics. X. Synthesis and structure-activity relationships of 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]cephalosporin derivatives. J. Antibiotics 39(1): 1986, in press
15) CLARK, J. C.; J. KENNEDY, A. G. LONG & N. G. WEIR: Antibiotic cephalosporin compounds. Ger. Offen. 2,103,014, July 29, 1971 [Chem. Abst. 75: 118328q]
16) TAKAYA, T.; H. TAKASUGI, T. MURAKAWA & H. NAKANO: Studies on β-lactam antibiotics. III. Synthesis and enzymatic stability of 3-acyloxymethyl-7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylic acids. J. Antibiotics 34: 1300~1310, 1981
17) FECHTIG, B.; H. PETER, H. BICKEL & E. VISCHER: 124. Modifikationen von Antibiotika. Über die Darstellung von 7-Amino-cephalosporansäure. Helv. Chim. Acta 51: 1108~1119, 1968
18) TAKASUGI, H.; H. KOCHI, T. MASUGI, H. NAKANO & T. TAKAYA: Studies on β-lactam antibiotics. VII. Effect on antibacterial activity of the oxime *O*-substituents with various functional groups in the 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-oxyiminoacetamido]cephalosporins. J. Antibiotics 36: 846~854, 1983
19) SHIGI, Y.; Y. MATSUMOTO, M. KAIZU, Y. FUJISHITA & H. KOJO: Mechanism of action of the new orally active cephalosporin FK027. J. Antibiotics 37: 790~796, 1984
20) SAKAMOTO, H.; T. HIROSE & Y. MINE: Pharmacokinetics of FK027 in rats and dogs. J. Antibiotics 38: 496~504, 1985
21) KAMIMURA, T.; H. KOJO, Y. MATSUMOTO, Y. MINE, S. GOTO & S. KUWAHARA: In vitro and in vivo antibacterial properties of FK 027, a new orally active cephem antibiotic. Antimicrob. Agents Chemother. 25: 98~104, 1984