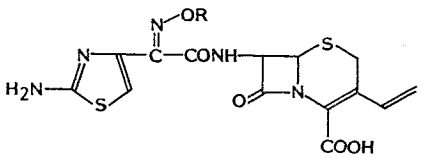
FK 482, A NEW ORALLY ACTIVE CEPHALOSPORIN SYNTHESIS AND BIOLOGICAL PROPERTIES

Sir:

Recently, we have reported the synthesis and biological properties of a new orally absorbable cephem, cefixime (CFIX, **1a**) and the related compounds[1~4]. CFIX is the first orally active cephem having an aminothiazolyl side chain in the 7-position like newer parenteral cephalosporins such as ceftizoxime and has a broad spectrum of antibacterial activity and a high stability against various β-lactamases. However, CFIX shows only low to moderate antibacterial activity against a few Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis*. In the course of our extensive research on orally active cephems, our efforts have been focused on synthesizing new cephalosporins with enhanced activity against such Gram-positive bacteria. As a result, we found a new orally active cephem, FK 482 (**1b**), 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic acid[5]. We here report the synthesis and the biological properties of FK 482.

FK 482 was synthesized as outlined in Scheme 1. Diphenylmethyl 7-amino-3-vinyl-3-cephem-4-carboxylate hydrochloride (**2**) was treated with 4-bromoacetoacetyl bromide in the presence of *N*-trimethylsilyl acetamide (EtOAc, −10°C, 1 hour) to give the acylated compound **3** in 88% yield. Nitrosation of **3** with aqueous sodium nitrite in dichloromethane and AcOH (−5°C, 30 minutes) following addition of urea to quench excess reagent gave the hydroxime compound **4** in quantitative yield. **4** was reacted with thiourea in *N*,*N*-dimethylacetamide (5°C, 1 hour) to give the thiazole derivative **5**. The crude product **5** was treated with TFA-anisole (5°C, 1 hour) followed by recrystallization from water to give FK 482 (**1b**) in 45% yield from **4**. The structure of **1b** was confirmed by the elemental analysis and ^{1}H NMR†. The chemical shift (6.65 ppm) of the annular proton at C-5 of the thiazole ring is consistent with the assignment of *Z* configuration[6].



Cefixime (**1a**) R = CH_2COOH
FK 482 (**1b**) R = H

Table 1 shows antibacterial spectrum of FK 482 as compared with those of CFIX, cefaclor (CCL) and amoxicillin (AMPC). FK 482 had a broader spectrum than those of the reference drugs. Against Gram-positive bacteria, FK 482 exhibited excellent activity. Against Gram-negative bacteria, it displayed much more potent activity than that of CCL and AMPC and comparable or slightly inferior to that of CFIX. However, it was inactive against *Pseudomonas aeruginosa*. The most remarkable feature of FK 482 is the excellent activity against staphylococcal species. Table 2 shows MIC_{50} and MIC_{80} against *S. aureus* and *Staphylococcus epidermidis* of clinical isolates. FK 482 exhibited the highest activity against methicillin-sensitive *S. aureus* (MSSA) and *S. epidermidis*, MIC_{80}: 0.39 and 0.78 μg/ml respectively. Moreover, FK 482 showed moderate activity (MIC_{50}: 6.25 and MIC_{80}: 12.5 μg/ml) against methicillin-resistant *S. aureus* (MRSA) although the reference drugs were inactive. In summary, FK 482 has excellent and well-balanced spectra against Gram-positive and Gram-negative bacteria except *P. aeruginosa*.

The urinary recovery of FK 482 in various animals is listed in Table 3. Considerable differences in recovery were observed among the animal species tested. Good oral absorption of FK 482 was observed in the rabbit and the dog although the absorption in the mouse and the rat was moderate.

† Analytical and ^{1}H NMR data of **1b** are as follows: *Anal* calcd for $C_{14}H_{13}N_5O_5S_2$: C 42.53, H 3.31, N 17.71, S 16.22. Found: C 42.23, H 3.18, N 17.63, S 16.31. ^{1}H NMR (90 MHz, DMSO-d_6) 3.53 and 3.80 (2H, ABq, J=18 Hz, SCH_2), 5.17 (1H, d, J=5Hz, 6-H), 5.28 (1H, d, J=10 Hz, $^{H}\!>\!C\!=\!C\!<^{H}_{H}$), 5.57 (1H, d, J=17 Hz, $^{H}\!>\!C\!=\!C\!<^{H}_{H}$), 5.75 (1H, dd, J=8 and 5 Hz, 7-H), 6.65 (1H, s, thiazole 5-H), 6.90 (1H, dd, J=17 and 10 Hz, $^{H}\!>\!C\!=\!C\!<^{H}_{H}$), 7.07 (2H, br s, NH_2), 9.42 (1H, d, J=8 Hz, NH), 11.25 (1H, br s, NOH).

Scheme 1.

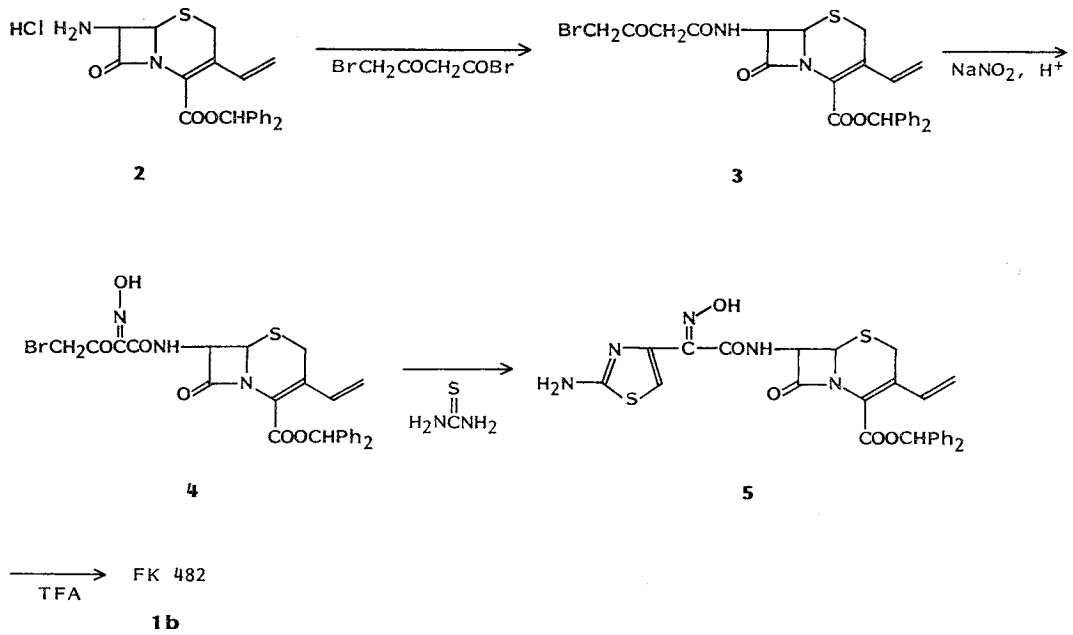


Table 1. Antibacterial spectrum of FK 482 and related antibiotics (MIC: μg/ml).

Organism	FK 482	CFIX	CCL	AMPC
Staphylococcus aureus 209P JC1	0.05	25	0.78	0.10
S. aureus 2535 (MRSA)	6.25	100	100	25
Streptococcus pyogenes S 23*	≦0.025	0.10	0.20	≦0.025
Siridans-type pneumoniae 4004*	≦0.025	0.05	0.39	≦0.025
Viridans-type streptococcus 3002*	0.39	1.59	12.5	0.39
Enterococcus faecalis 115	6.25	>100	>100	0.78
Neisseria gonorrhoeae PCL783*	≦0.025	≦0.025	0.05	0.20
Haemophilus influenzae 57*	0.20	≦0.025	1.56	0.20
Escherichia coli NIHJ JC2	0.10	0.10	3.13	3.13
Klebsiella pneumoniae NCTC 418	0.10	≦0.025	0.78	25
Proteus mirabilis 1	0.10	≦0.025	1.56	0.78
P. vulgaris IAM 1025	0.20	≦0.025	6.25	6.25
Citrobacter freundii 3029	3.13	3.13	25	>100
Serratia marcescens 3049	6.25	0.39	>100	50
Pseudomonas aeruginosa IAM 1095	>100	100	>100	>100
Bacteroides fragilis Ju13	1.56	3.13	25	1.56

Müller-Hinton agar; 10^{-2}, stamp method; 37°C, 20 hours.
* Supplemented with 5% horse blood.
CFIX: Cefixime, CCL: cefaclor, AMPC: amoxicillin.

The good oral absorption of FK 482 could be anticipated from the structural similarity to CFIX; only the oxime moieties in structure **1** differ from each other. In addition, the oximes of both compounds are acidic functional groups as a common property, although the difference in acidity between each group is considerable†.

Consequently, on the basis of its excellent antibacterial activity together with its good oral

† The *pKa* values of the hydroxyimino group of FK 482 and the carboxymethoxyimino group of CFIX are 9.70 and 3.73, respectively.

Table 2. MIC_{50} and MIC_{80} of FK 482 and related antibiotics against *Staphylococcus* sp. (MIC: μg/ml).

Organism (No. of strains)	FK 482		CFIX		CCL		AMPC	
	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}	MIC_{80}
Staphylococcus aureus; MSSA (54)	0.39	0.39	12.5	25	1.56	3.13	0.2	0.39
S. aureus; MRSA (24)	6.25	12.5	>100	>100	>100	>100	50	>100
S. epidermidis (49)	0.1	0.78	6.25	>100	1.56	12.5	0.39	6.25

Müller-Hinton agar; 10^{-2}, stamp method; 37°C, 20 hours.
CFIX: Cefixime, CCL: cefaclor, AMPC: amoxicillin.

Table 3. Urinary and biliary excretion after an oral dose of 20 mg/kg.

	Recovery (%) (in 24 hours)			
	Mouse	Rat	Rabbit	Dog
Urinary	9.8	15.5	45.8	47.1
Biliary	—	1.4	—	—

Animal: Mouse; ICR strain (male, 4W old), 10 mice/group, Rat; SD strain (male, 6W old), 5~10 rats/group, Rabbit; Japanese white rabbit (male, 2.6~2.8 kg), 5 rabbits/group, Dog; Beagle (male, 9.5~12.0 kg), 4~5 dogs/group.
—: Not tested.

absorption in animals, FK 482 (**1b**) was selected for further evaluation. These results including that of human trials will be published elsewhere.

Acknowledgment

The authors wish to thank Drs. Y. MINE and K. SAKANE for helpful discussions in preparing this paper.

YOSHIKO INAMOTO
TOSHIYUKI CHIBA
TOSHIAKI KAMIMURA
TAKAO TAKAYA*

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

(Received January 5, 1988)

References

1) 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 acid. J. Antibiotics 38: 1068~1076, 1985
2) YAMANAKA, H.; T. CHIBA, K. KAWABATA, H. TAKASUGI, T. MASUGI & T. TAKAYA: Studies on β-lactam antibiotics. IX. Synthesis and biological activity of a new orally active cephalosporin, cefixime (FK027). J. Antibiotics 38: 1738~1751, 1985
3) 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: 101~110, 1986
4) KAWABATA, K.; H. YAMANAKA, H. TAKASUGI & T. TAKAYA: Studies on β-lactam antibiotics. XIII. Synthesis and structure-activity relationships of 7β-[(*Z*)-2-aryl-2-carboxymethoxyimino-acetamido]-3-vinylcephalosporins. J. Antibiotics 39: 404~414, 1986
5) TAKAYA, T.; T. KAMIMURA, Y. WATANABE, Y. MATSUMOTO, S. TAWARA, F. SHIBAYAMA, Y. MINE & S. KUWAHARA: FK482, a new orally active cephalosporin: In vitro antibacterial activity. Program and Abstracts of the 27th Intersci. Conf. on Antimicrob. Agents Chemother., No. 652, p. 210, New York, Oct. 4~7, 1987
6) OCHIAI, M.; A. MORIMOTO, Y. MATSUSHITA & T. OKADA: Synthesis and structure-activity relationships of 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporin derivatives. IV. Synthesis of 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid derivatives and related compounds. J. Antibiotics 34: 160~170, 1981