COMMUNICATIONS TO THE EDITOR

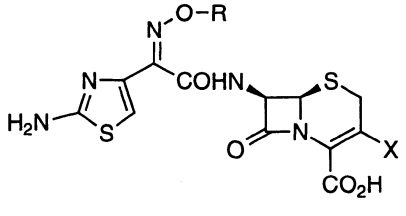
FK041, a Novel Orally Active Cephalosporin: Synthesis and Biological Properties

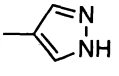
Sir:

Recently, new oral cephalosporins with broad spectra of activity and high stability against various β-lactamases, such as cefixime[1] (CFIX), ceftibutene[2] (CFTB), and cefdinir[3] (CFDN), have been developed and introduced to clinical practice. In particular, CFDN, which was discovered in our laboratories, has excellent antibacterial activity against both Gram-positive and -negative bacteria and high oral absorption.[3] CFDN has shown excellent clinical efficacy and is extremely well tolerated by patients and has become the most widely used oral cephalosporin in Japan since its approval in 1991. However, CFDN exhibits relatively low efficacy against *Haemophilus influenzae*, which is a very important pathogen leading to respiratory infections. Thus, our next research objective was to discover a new cephalosporin having more potent activity against *H. influenzae* and equal, or higher oral absorption than CFDN without using a prodrug strategy. We focused our attention on modification of the substituent at the C-3 position of CFDN, since the (*Z*)-2-(2-amino-4-thiazolyl)-2-hydroxyiminoacetyl side chain is critical for displaying activity against *Staphylococcus aureus*, and for the high oral absorption of CFDN.[4] As a result, we have discovered FK041[5,6] (Fig. 1) with a novel 4-pyrazolylmethylthio moiety that displays both very potent activity against *H.influenzae* and higher oral absorption. We report here the synthesis and biological properties of FK041.

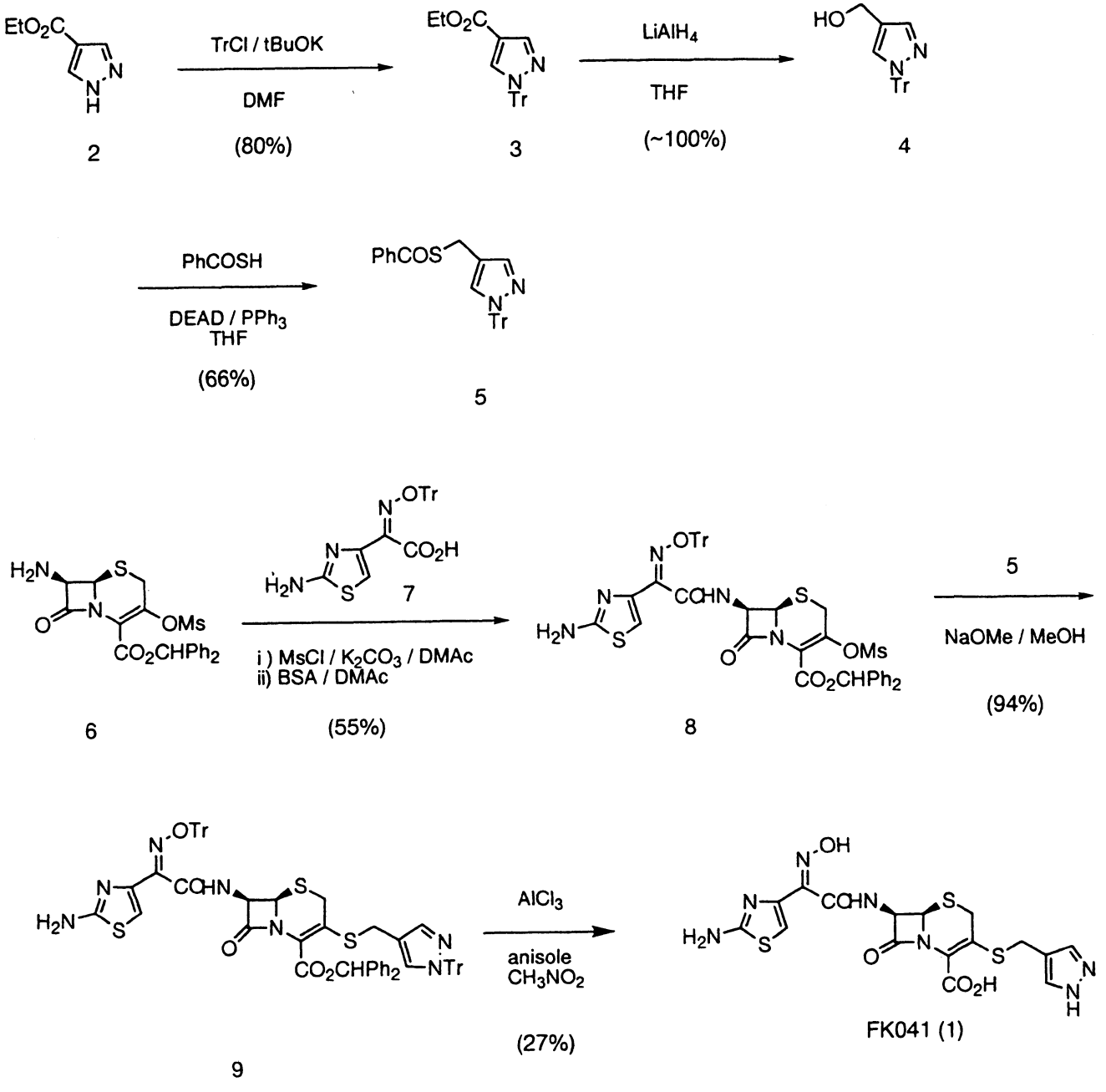
The synthesis of FK041 is outlined in Scheme 1. The key intermediate for the C-3 side chain, 4-benzoylthiomethyl-1-tritylpyrazole (**5**) was prepared in 3 steps from the commercially available 4-ethoxycarbonylpyrazole (**2**). Protection of **2** with tritylchloride, followed by reduction with lithium aluminium hydride and Mitsunobu reaction with *S*-thiobenzoic acid in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine afforded **5**. 7β-Acylcephem (**8**) was prepared by coupling reaction of 2-(2-amino-4-thiazolyl)-2-(*Z*)-trityloxyiminoacetic acid (**7**), activated with methanesulfonyl chloride, and 7β-amino-3-methanesulfonyloxycephem (**6**)[7] in the presence of bis(trimethylsilyl)acetamide (BSA) in good yield. The mesyl group at the 3-position of **8** was directly displaced by the thiolate anion generated *in situ* from **5** in the presence of sodium methoxide to afford a mixture of protected Δ^3 cephem (**9**) and the corresponding Δ^2 cephem. The major product was the desired Δ^3 isomer (**9**) and the small amounts of the Δ^2 isomer were readily removed by silica-gel chromatography. Subsequent deblocking of **9** was effected by treatment with aluminium chloride in nitromethane to afford FK041 (**1**). The structure of FK041 was confirmed on the basis of IR, NMR, FAB-MS and elemental

Fig. 1. Structures of CFIX, CFDN and FK041.



	R	X
CFIX	—CH_2CO_2H	—$CH{=}CH_2$
CFDN	—H	—$CH{=}CH_2$
FK041(1)	—H	—SCH_2— 

Scheme 1. Synthesis of FK041 (1).



analysis data (Table 1).

The antibacterial activity (MIC_{80}) of FK041 is shown in Table 2. For comparison, the MIC_{80} values for CFDN and cefditoren[8] (CDTR) are also listed. FK041 showed the most potent activity against *S. aureus* of the three drugs, and also showed potent activity against PC-susceptible *Streptococcus pneumoniae*. In addition to reasonable activity against PC-resistant *S. pneumoniae*, FK041 also showed moderate activity against *Enterococcus faecalis*, although CDTR was inactive. Against Gram-negative bacteria, FK041 was more active than CFDN. In particular, the activity against *H. influenzae* of FK041 was 3-fold superior to that of CFDN. Compared with CDTR, FK041 showed more potent activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. As a result, FK041 had excellent and well-balanced activity against both Gram-positive and -negative bacteria, and superior activity against *H. influenzae* compared with CFDN.

Table 3 shows the comparative pharmacokinetics and urinary and biliary recovery after oral administration (20 mg/kg) of FK041, CFDN and CDTR-PI to various

Table 1. IR, NMR, FAB-MS and analytical data of FK041.

IR (KBr) cm^{-1}	1763, 1647, 1603, 1541
^1H NMR (200 MHz, DMSO-d_6) δ	3.69 and 3.74 (2H, ABq, J=14.2 Hz, C2-H_2), 3.99 and 4.06 (2H, ABq, J=13.4 Hz, $-CH_2$-pyrazole), 5.15 (1H, d, J=4.6 Hz, C6-H), 5.69 (1H, dd, J=8.2, 4.6 Hz, C7-H), 6.71 (1H, s, thiazole-5H), 7.30 (2H, s, thiazole NH_2), 7.56 (2H, s, pyrazole-3, 5H), 9.48 (1H, d, J=8.2 Hz, CONH), 11.41 (1H, s, NOH)
FAB-MS: m/z	481 (M^+).
Analysis	
Calcd for $C_{16}H_{15}N_7O_5S_3 \cdot 3.75H_2O$:	C 35.00, H 4.13, N 17.86, S 17.52
Found:	C 34.71, H 3.84, N 17.79, S 17.30

Table 2. Antibacterial activity of FK041 and reference antibiotics.

Drugs	MIC_{80} (μg/ml)								
	S. a	*S. p.* 1	*S. p.* 2	*E. f.*	*M. c.*	*H. i.*	*E. c.*	*K. p.*	*P. m.*
FK041	0.39	0.1	3.13	25	0.39	0.2	0.1	0.05	0.05
CFDN	0.78	0.2	6.25	100	0.39	1.56	0.39	0.2	0.1
CDTR	1.56	0.1	0.78	>100	0.2	0.05	0.39	0.39	0.1

Müller-Hinton agar; 10^{-2}, stamp method; 37°C, 20 hours.
S. a., *Staphylococcus aureus* (MSSA) (40); *S. p.* 1, *Streptococcus pneumoniae* (PC-susceptible) (33); *S. p.* 2, *Streptococcus pneumoniae* (PC-resistant) (48); *E. f.*, *Enterococcus faecalis* (20); *M. c.*, *Morachisella catarrhalis* (21); *H. i.*, *Haemophilus influenzae* (83); *E. c.*, *Escherichia coli* (21); *K. p.*, *Klebsiella pneumoniae* (20); *P. m.*, *Proteus mirabilis* (20).
CFDN: cefdinir, CDTR: cefditoren.

Table 3. Comparative pharmacokinetics of FK041 and reference antibiotics after single oral administration (20 mg/kg) to various animals.

Animal	Drugs	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC (μg·h/ml)	Recovery (%)	
					Urine	Bile
Mouse	FK041	2.6	1.9	8.3	17.1	11.1
	CFDN	2.2	1.6	6.2	35.5	1.49
	CDTR-PI	8.7	1.2	14.8	1.94	12.2
Rat	FK041	7.1	1.4	28.0	42.9	6.81
	CFDN	2.1	0.8	6.1	32.5	1.40
	CDTR-PI	13.9	1.7	36.5	5.75	16.8
Rabbit	FK041	13.2	1.4	40.5	48.8	nd
	CFDN	5.28	1.11	14.5	45.8	nd
	CDTR-PI	nd	nd	nd	nd	nd
Dog	FK041	13.6	1.4	45.0	27.8	nd
	CFDN	43.1	3.4	413.9	41.3	0.013
	CDTR-PI[a]	0.9	0.9	2.7	nd	nd

[a] T. MATSUMOTO *et al.* 1992. Chemotherapy (Tokyo). 40 (S-2): 120~130.
nd: not determined.

Table 4. Protective effect of FK041 and CFDN on systemic infection in mice.

Organism	Challenge dose (CFU/mouse, i.p.)	Drug	ED_{50} (mg/kg)	MIC (μg/ml)
S. aureus Smith	2.6×10^7	FK041	0.35	0.39
		CFDN	0.9	0.78
E. coli 29	2.0×10^6	FK041	0.36	$\leqq$0.025
		CFDN	2.2	0.1
K. pneumoniae 1	1.5×10^5	FK041	0.2	0.1
		CFDN	1.3	0.2

Treatment: p.o., 1 hour after challenge.

animals. FK041 showed little variability in pharmacokinetics and recovery among the tested animals. In mouse, pharmacokinetics and recovery of FK041 were similar to CFDN. In rat, FK041 showed the best recovery of the three compounds. In rabbit, FK041 showed the most superior data in terms of both pharmacokinetics and recovery. In dog, pharmacokinetics and recovery of FK041 were inferior to those of CFDN, however, CFDN shows exceptionally good data in dogs. As a result, the pharmacokinetics and recovery of FK041 were judged to be good in all animals.

We further tested *in vivo* activity of FK041 against systemic infections with *S. aureus* Smith, *E. coli* 29 and *K. pneumoniae* 1 in mice and results are shown in Table 4. The *in vivo* efficacy of FK041 in an experimental infection due to *S. aureus* Smith was superior to that of CFDN. In experimental infections caused by *E. coli* 29 and *K. pneumoniae* 1, FK041 exhibited 6 times greater *in vivo* efficacy than CFDN. As a consequence FK041 showed greater *in vivo* activity than CFDN, and this *in vivo* efficacy reflected the MIC values.

In conclusion, FK041 exhibited potent activity against *H. influenzae* together with well-balanced activity against both Gram-positive and -negative bacteria, as well as good oral absorption, pharmacokinetics and high *in vivo* efficacy. Therefore, FK041 was selected as a clinical candidate. The results of pharmacokinetics and tolerance of FK041 in healthy volunteers will be published elsewhere.

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