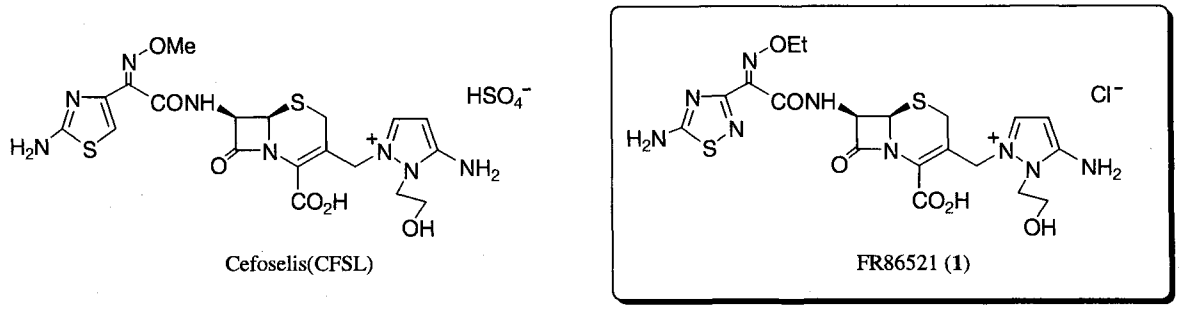
Synthesis and Biological Properties of a Novel Cephalosporin FR86521 Having Potent Activity Against Methicillin-resistant *Staphylococcus aureus* (MRSA)

Sir:

In the last decade, various parenteral 3′-quarternary ammonium cephalosporin derivatives with a broad spectrum of antibacterial activity have been investigated and marketed, such as cefpirome (CPR)[1], cefepime (CFPM)[2] and cefozopran (CZOP)[3]. Nevertheless, antibiotics having low toxicity and high activities against both Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria including *Pseudomonas aeruginosa* (*P. aeruginosa*), which are the main bacteria causing nosocomial infections, are now urgently required. Recently, we introduced cefoselis (CFSL)[4,5], which shows potent broad-spectrum activity against both Gram-positive bacteria, including MRSA, and Gram-negative bacteria, including *P. aeruginosa*. CFSL has a 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido] side chain. We have focused recent efforts on synthesizing novel cephalosporins superior to CFSL in antibacterial activity against both MRSA and *P. aeruginosa*, and as a result, we have discovered FR86521 (**1**)[6] having a 7β-[(*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-ethoxyiminoacetamido] side chain (Fig. 1). In this paper, we report the synthesis, and *in vitro*

Fig. 1.



Scheme 1.

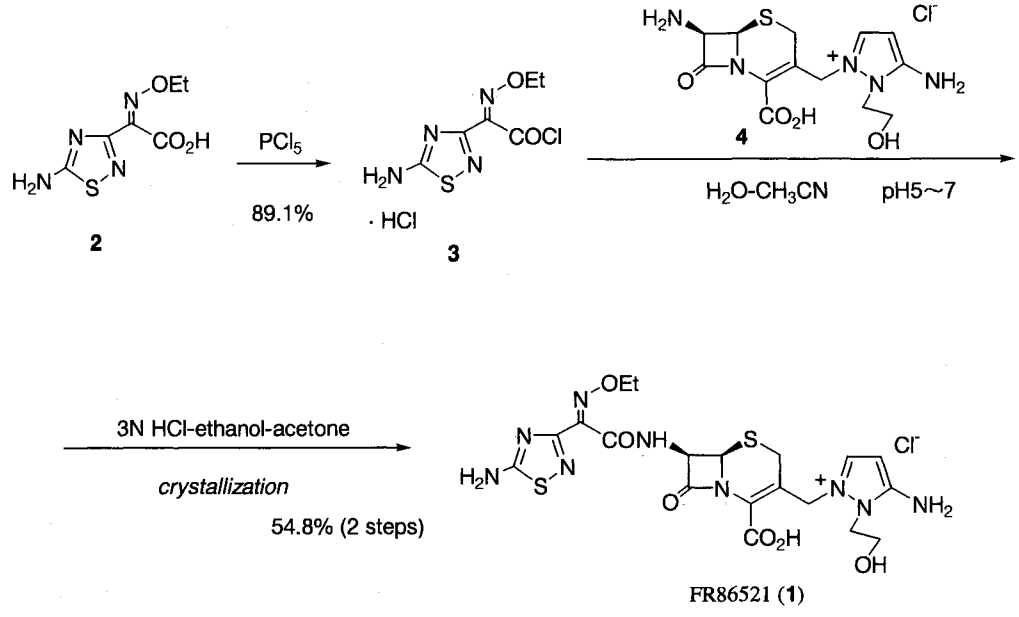


Table 1. IR, ^{1}H NMR, MS and analytical data of FR86521.

IR (KBr) cm^{-1}	3298, 1788, 1659, 1641, 1039
^{1}H NMR (200MHz, DMSO-d_6) δ	1.24 (3H, t, J=7.1Hz, OCH_2CH_3), 3.25 and 3.35 (2H, ABq, J= 18.3Hz, 2-H_2), 3.50~3.70 (2H, m, NCH_2CH_2OH), 4.17 (2H, q, J=7.1Hz, OCH_2CH_3), 4.00~4.20 (1H, m, HCHOH), 4.30~4.50 (1H, m, HCHOH), 5.14 and 5.33 (2H, ABq, J= 16.0Hz, 3'-H_2), 5.19 (1H, d, J=4.9Hz, 6-H), 5.87 (1H, dd, J= 8.5 and 4.9Hz, 7-H), 5.90 (1H, d, J= 3.3Hz, pyrazole 4-H), 7.51 (2H, s, pyrazole NH_2) , 8.04 (1H, d, J=3.3Hz, pyrazole 5-H), 8.22 (2H, s, thiadiazole NH_2), 9.60 (1H, d, J=8.5Hz, CONH)
MS: m/z	538.2(M-HCl)
Analysis	
Calcd for $C_{19}H_{24}ClN_9O_6S_2 \cdot 2H_2O$:	C 37.41, H 4.63, Cl 5.81, N 20.66, S 10.51
Found:	C 37.32, H 4.36, Cl 6.11, N 20.55, S 10.29

Table 2. Spectrum of antibacterial activity of FR86521.

Organism (no. of strains) \ Drug		**FR86521**	CFSL	CZOP	FMOX	CAZ
S. aureus	(9)	1.06	0.78	0.78	0.39	8.5
MRSA	(9)	4.6	27	43	37	>100
E. faecalis	(9)	31	100	13.5	>100	>100
S. pyogenes	(9)	0.0132	0.0063	0.023	N.D.	N.D.
S. pneumoniae	(9)	0.033	0.031	0.072	N.D.	N.D.
PRSP	(9)	0.72	0.72	1.45	N.D.	N.D.
B. catarrhalis	(9)	0.167	0.155	0.62	0.052	0.048
E. coli	(9)	0.041	<0.025	0.035	0.035	0.09
K. pneumoniae	(9)	0.041	0.03	0.035	0.035	0.048
P. mirabilis	(9)	0.155	0.03	0.143	0.143	0.038
P. vulgaris	(9)	0.42	0.083	0.84	0.21	0.035
S. marcescens	(9)	1.69	0.84	0.98	18.4	0.84
E. cloacae	(9)	0.29	0.18	0.195	34	1.45
C. freundii	(9)	0.36	0.39	0.31	25	6.8
P. aeruginosa	(9)	1.82	6.8	1.97	>100	2.7
CPZ-R	(9)	11.6	43	17.0	>100	5.8
CAZ-R	(9)	15.7	>100	37	>100	54
IPM-R	(9)	5.0	19.8	7.9	>100	9.2

geometric mean MIC, μg/ml, Inoculum size: 10^{-2} N.D. : Not determined

Table 3. Antibacterial activity against clinical isolates of FR86521.

Organism (no. of strains)	Inoculum size	Drug	MIC (μg/ml) distribution	geometric mean	50%	90%
MRSA (27)	10^{-2}	**FR86521**	1.56~ 6.25	4.25	6.25	6.25
		CFSL	1.56~ 25	11.5	12.5	12.5
		VCM	0.78~ 1.56	0.87	0.78	1.56
	10^{0}	**FR86521**	1.56~ 6.25	5.79	6.25	6.25
		CFSL	1.56~ 50	20.4	25	25
		VCM	1.56~ 6.25	2.89	3.13	3.13

Table 4. Protective effect against MRSA on systemic infection in mice of FR86521.

Strain (Inoculum; CFU/mouse)	Drug	ED_{50} (mg/kg)	MIC (μg/ml) (10^{-2})	(10^{0})
S. aureus 8008 ($2.1x10^{8}$)	**FR86521**	3.29	6.25	6.25
	VCM	2.25	0.78	3.13
S. aureus 5027 ($2.5x10^{8}$)	**FR86521**	5.28	6.25	N.D.
	VCM	6.48	0.78	N.D.

mouse: ICR strain, male, 4 weeks, n=8
N.D. : Not determined

Table 5. Protective effect against *P. aeruginosa* on systemic infection in mice of FR86521.

Strain (Inoculum; CFU/mouse)	Drug	ED_{50} (mg/kg)	MIC (μg/ml) (10^{-2})	(10^{0})
P. aeruginosa 93 ($1.1x10^{6}$)	**FR86521**	1.57	0.78	1.56
	CZOP	3.49	0.78	1.56
	CAZ	30.0	1.56	1.56
P. aeruginosa 4055 ($1.5x10^{6}$)	**FR86521**	6.13	3.13	N.D.
	CAZ	28.7	12.5	N.D.

mouse: ICR strain, male, 4weeks, n=8 N.D. : Not determined

and *in vivo* antibacterial activity against MRSA and *P. aeruginosa* of FR86521.

The synthesis of FR86521 is outlined in Scheme 1. (*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-ethoxyiminoacetyl chloride hydrochloride (**3**) was prepared from the corresponding carboxylic acid (**2**) with phosphorus pentachloride in 89.1% yield[7]. 7β-Aminocephalosporin derivative (**4**) having the aminopyrazoliomethyl moiety at the 3-position[5] was acylated with the acyl chloride (**3**) under Schotten acylation conditions and crystallized

with 3 N hydrochloric acid in ethanol-acetone to produce FR86521 in 54.8% yield. The structure of FR86521 was confirmed by IR, ^{1}H NMR, MS and elemental analysis (Table 1).

The antibacterial activity (mean MICs) of FR86521 along with CFSL, CZOP, flomoxef (FMOX) and ceftazidime (CAZ) as reference compounds against selected Gram-positive and Gram-negative bacteria, are shown in Table 2. FR86521 showed extremely potent broad-spectrum activity against both Gram-positive bacteria and Gram-negative bacteria. Especially, the activity of FR86521 against MRSA was at least 6-fold greater than the reference cephalosporins. Moreover, FR86521 had the highest activity against *P. aeruginosa*, including CAZ and imipenem (IPM) resistant strains. Thus, FR86521 exhibited excellent activity against both MRSA and *P. aeruginosa*, which are at the center of particular concern, since they cause severe nosocomial infections.

The antibacterial activities (MIC_{50}, MIC_{90}) of FR86521, CFSL and vancomycin (VCM) against clinically isolated MRSA with varying inoculum size, are shown in Table 3. Whilst the activity of VCM decreased compared with larger inoculum size, the activity of FR86521 was not influenced. Thus, FR86521 displayed better bactericidal activity than VCM against MRSA.

The protective effects of FR86521 and VCM against systemic infection by MRSA (*S. aureus* 8008 and *S. aureus* 5027) in mice are shown in Table 4. The efficacy of each compound was expressed as the 50% effective dose value (ED_{50}) calculated by the probit method. The ED_{50} value of FR86521 against both MRSA strains was nearly equal to that of VCM. Thus FR86521 was the most effective drug against MRSA among the reference cephalosporins.

The protective effects of FR86521 and the reference drugs (CZOP, CAZ) against systemic infection by *P. aeruginosa* (93 and 4055) in mice are shown in Table 5. The ED_{50} value of FR86521 against both *P. aeruginosa* strains was the most effective of the reference drugs, including CZOP.

In summary, FR86521 having a 5-amino-1,2,4-thiadiazol-3-yl side chain at the 7-position exhibited more potent *in vitro* and *in vivo* activity against MRSA and *P. aeruginosa* compared with marketed cephalosporins. Moreover, FR86521 showed the same *in vivo* efficacy against MRSA as VCM. Further evaluation of FR86521 will be published in subsequent papers.

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