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Synthesis and SAR of novel parenteral anti-pseudomonal cephalosporins: Discovery of FR264205

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ABSTRACT

We describe herein the synthesis and biological evaluation of a series of novel cephalosporins with potent activity against Pseudomonas aeruginosa. Introduction of various amino groups to the 4-position of a 3-amino-2-methylpyrazole cephalosporin 3-side chain resulted in enhanced MIC values against multiple *Pseudomonas aeruginosa* strains and ultimately led to the discovery of FR264205 (**15**) with excellent anti-bacterial activity and weak convulsion effect by direct intracerebroventricular injection assay.

Pseudomonas aeruginosa is a major nosocomial pathogen and is responsible for many serious infections in patients who are immunocompromised due to treatment for malignant tumors, neutropenia or resulting from organ transplantation.[1] Such infections are difficult to cure, especially with the ongoing emergence of strains resistant to the currently available clinically used agents, which often leads to prolongation of the treatment period. Against this bacterium, several members of the carbapenem class of antibacterials have been effective, but their use has induced resistance due to decreased penetration through the outer membrane resulting from loss of the D2 porin.[2] Certain fluoroquinolones also show potent anti-pseudomonal activity, but some strains have acquired resistance due to mutations of the drug target and/or due to induction of active efflux pumps.[3] In terms of general resistance trends, whilst much recent research has been focused on tackling the problems of resistant Gram-positive pathogens such as MRSA and VRE, only few efforts have been directed at identifying improved agents for *P. aeruginosa* infections.

Among the marketed cephalosporins, Ceftazidime (CAZ) shows the best anti-pseudomonal activity, but displays very little activity against AmpC β-lactamase-producing strains which have recently been increasing and are becoming a significant problem.[4] During the course of our research on fourth-generation broad-spectrum cephalosporins, we previously discovered FK037 (cefoselis)[5] and FK518 (Fig. 1).[6] Although both compounds possess an aminopyrazole ring at the 3-position of the cephalosporin nucleus, the distinctive chemical feature of FK518 is the presence of a dimethylacetic acid group in place of the methyl group on the oxime moiety and a thiadiazole instead of thiazole at the 7-position. These modifications were presumed to be responsible for the improved efficacy of FK518 against *P. aeruginosa* species.

In the research described herein, we report our efforts aimed toward the discovery of novel cephalosporin derivatives with potent activity against Class C (AmpC) β-lactamase-producing *P. aeruginosa* strains and a low convulsion-inducing effect, and the discovery of FR264205 as a novel agent with significantly improved activity and good potential as a new anti-pseudomonal agent.[7]

In general terms, a strategy to improve the anti-pseudomonal activity of cephem derivatives requires either, or all of, the following: (1) maintenance of high penicillin-binding protein (PBP) affinity, (2) increased outer membrane permeability, and (3) improved stability to AmpC β-lactamase. Our previous study showed that the PBP affinity of FK518 was very high, especially toward PBP3 which is important for anti-bacterial activity, presumably due to the contribution of the dimethylacetic acid moiety on the oxime at the 7-position.[6] This speculation was supported by the PBP affinity data of CAZ[8] (which also has dimethylacetic acid moiety) which is higher compared to FK037 and CZOP (with a 7-position methoxyimino moiety). Therefore, we selected this structure for our investigations and our attention was focused on the following strategy.

The outer membrane of *P. aeruginosa* represents a formidable barrier to entry of antibiotics, and the relatively large side chain at the 7-position was presumed to be unfavorable for outer-mem-

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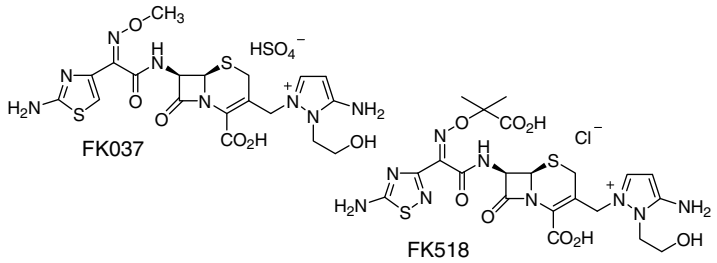
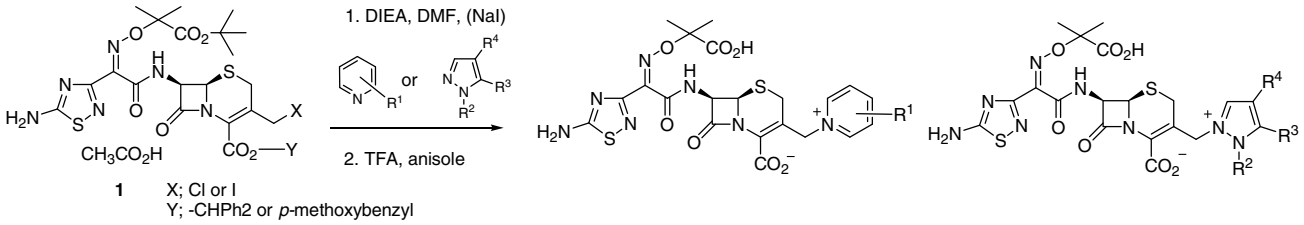
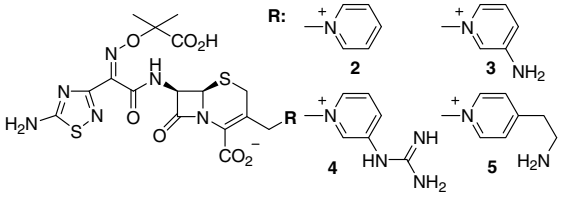


Figure 1. Structures of FK037 (cefoselis) and FK518.



Scheme 1. Synthesis of novel cephalosporins.

Table 1
Anti-pseudomonal activities and permeability (differential ratio) of pyridiniumcephems



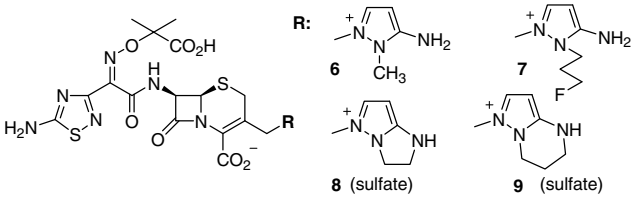
Compound	MIC (μg/mL)					Calculated pK_a (amine)
	FP2056[a]	*FP1380*[b]	mean[c]	MIC_{50}[c]	Permeability[d]	
2	1	32	2.1	2	NT	—
3	1	32	1.71	1	32	Non-basic
4	1	8	1.43	1	16	7.04
5	1	4	1.36	1	8	8.51
FK518	1	16	1.51	1	64	Non-basic

NT, not tested.
[a] Class A TEM β-lactamase-induced clinical isolate.
[b] Class C AmpC(ld) β-lactamase-induced clinical isolate.
[c] Fifty-four clinical isolates.
[d] $MIC_{DEAE\text{-}Dex(-)}/MIC_{DEAE\text{-}Dex(+)}$.

brane permeability. Our attention was initially focused on the observation that the outer-membrane permeability of carbapenems is good, and is most likely due to the small molecular size and the presence of a basic side chain.[9] We aimed to apply the latter observation to cephems by introducing various basic side-chains to the 3-position of the cephem nucleus. Initially, we introduced basic substituents to a pyridine ring to obtain a rapid validation of our hypothesis regarding outer membrane permeability. Basicity (pK_a) of side chains was calculated by ACD/PhysChem Batch Ver9 (ACD/Labs). Compounds **2–5** were synthesized according to the methods outlined in scheme 1. A chloromethyl (or iodomethyl) cephem derivative **1** was coupled with a pyridine derivative, followed by global deprotection and purification to afford the final cephem derivatives.

The *in vitro* antibacterial activity and outer-membrane permeability of compounds **2–5** is shown in Table 1. MICs were determined according to the CLSI method,[10] permeability was determined by differential assay from the ratio of MIC values in the presence and absence of 100 μg/mL DEAE-Dextran.[11] When an amino group is added to the 3-position of the pyridine side

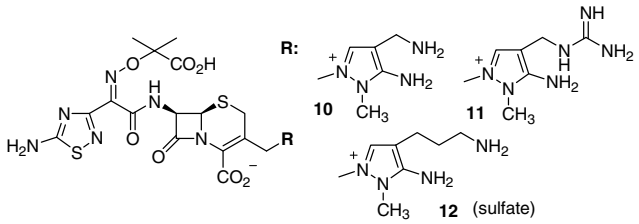
Table 2
Anti-pseudomonal activities of 2-modified pyrazoliocephems



Compound	MIC (μg/mL)			
	FP2056[a]	*FP1380*[b]	Mean[c]	MIC_{50}[c]
6	1	32	1.24	1
7	1	8	1.74	1
8	1	16	1.47	1
9	1	4	1.43	1
FK518	1	16	1.51	1

[a] Class A TEM β-lactamase-induced clinical isolate.
[b] Class C AmpC(ld) β-lactamase-induced clinical isolate.
[c] Fifty-four clinical isolates.

Table 3
Anti-pseudomonal activities and convulsion-inducing effect of 4-modified pyrazoliocephems



Compound	MIC (μg/mL)				Convulsion[d] ED_{50} (μg/head)	Calculated pK_a (amine)
	FP2056[a]	*FP7380*[b]	Mean[c]	MIC_{50}[c]		
10	0.5	8	0.91	0.5	39.3	7.27
11	0.5	1	0.66	0.5	4.69	10.66
12	0.5	1	0.77	0.5	80.1	9.66
FK518	1	16	1.51	1	46.9	Non-basic
CAZ	2	128	3.7	2	71	—
CZOP	2	32	2.7	2	230	—

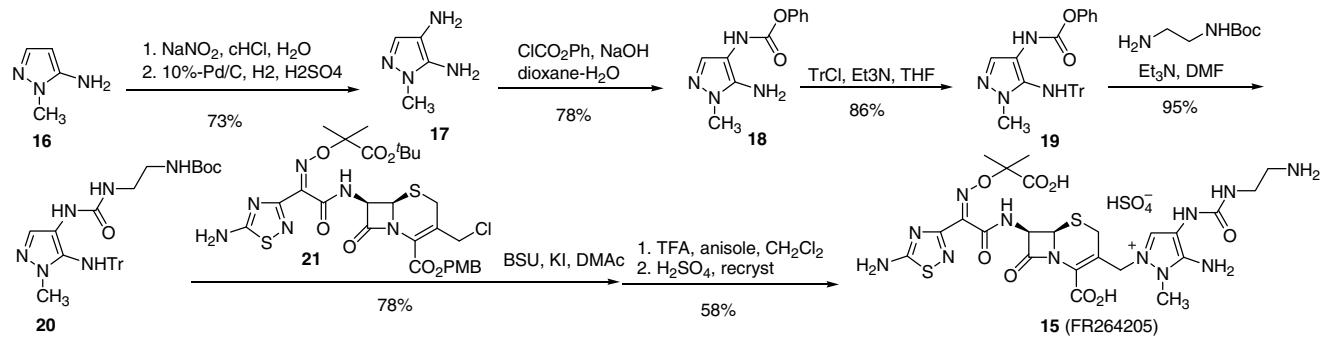
[a] Class A TEM β-lactamase-induced clinical isolate.
[b] Class C AmpC(ld) β-lactamase-induced clinical isolate.
[c] Fifty-four clinical isolates
[d] Convulsion-inducing effect: ED_{50} (μg/head, mouse intracerebroventricular injection).

chain (compound **3**), an improvement in activity relative to the non-substituted pyridine analog **2** was observed: mean MIC:1.71 vs 2.1 μg/mL, MIC_{50} 1 μg/mL vs 2 μg/mL (MIC_{50} = concentration inhibiting 50% of strains). Larger substituents such as a guanidino group (compound **4**) or an aminoethyl group (compound **5**) were tolerated and they showed improved activity against *P. aeruginosa*. As we had speculated, the presence of a basic side chain on the 3-position substituent appears to improve permeability. The permeability was improved as the basicity increased, with the best activity being observed for the 4-aminoethyl-substituted derivative **5**, with a calculated pK_a of 8.51 (Table 1). Although the MICs of the pyridine analogs **2–5** against *P. aeruginosa* FP2056, a Class A β-lactamase-inducer clinical isolate, were the same (1 μg/mL), those against *P. aeruginosa*. FP1380, a Class C β-lactamase inducer clinical isolate, were improved in parallel with the improved outer-membrane permeability, and this improved activity against Class C β-lactamase-producing strains led to an excellent mean MIC value (1.36 μg/mL; 54 clinically isolated strains).

The observed improvement in activity for the pyridine derivatives led us to speculate that further modification of the pyrazole moiety of FK518 may lead to clinically significant levels of anti-pseudomonal activity, since the intrinsic antibacterial activity of FK518 is superior to the pyridine derivatives. First, we introduced various substituents to the 2-position of the pyrazole ring to determine the most suitable structure for further investigation and optimization (Table 2). Compounds (**6–9**) were prepared as shown in Scheme 1 and evaluated for antibacterial activity.

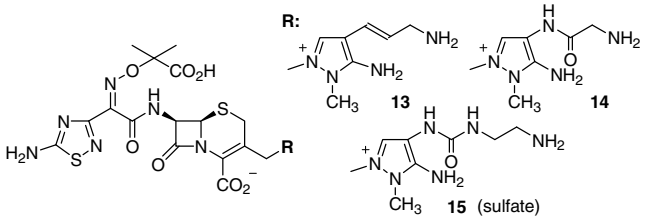
The structure of the substituent at the 2-position of the 3′-aminopyrazolium group affected MICs against the Class C β-lactamase-producing isolate, whilst MICs against the ClassA β-lactamase isolate were all the same (1 μg/mL). Compound **6** having a small substituent at this position showed weaker activity against Class C β-lactamase-producing strains than FK518 (32 and 16 μg/mL, respectively). This indicated that compound **6** was less stable to Class C (AmpC) β-lactamase. Relatively large substituents at this position (**7–9**) had a good effect on activity against the Class C β-lactamase-producing isolate (MICs: 8, 16, and 4 μg/mL, respectively). It is notable that modification of this part of the 3-position side chain can alter stability toward Class C β-lactamase. Therefore steric effects at this position appear to be important for stability. Although compound **6** only had a moderate MIC against the Class C-producing isolate, it had the best mean MIC (1.24 μg/mL). Therefore, we selected the 2-methylpyrazole group for further modification, and next introduced various basic side chains at the 4-position of the pyrazole ring (Table 3).

Compound **10** was obtained as indicated in our previous publication.[12] Compounds **11** and **12** were obtained as outlined in Scheme 1. Compound **10**, in which the aminomethyl group (pK_a = 7.27) is added to the 4-position of **6**, showed significantly improved anti-pseudomonal activity. MIC against the Class C β-lactamase-producing strain was 8 μg/mL for **10**, as compared to 32 μg/mL for **6**. The guanidino derivative **11** was even more potent, with an MIC of 1 μg/mL, presumably as a consequence of the high pK_a value (10.66). However, this compound (**11**) had very strong



Scheme 2. Synthesis of FR264205 (**15**).

Table 4
Anti-pseudomonal activities and convulsion-inducing effect of 4-modified pyrazoliocephems



Compound	MIC (μg/mL)				Convulsion[d] ED_{50} (μg/head)	Calculated pK_a (amine)
	FP2056[a]	*FP1380*[b]	Mean[c]	MIC_{50}[c]		
13	1	2	0.93	0.5	122	8.55
14	0.5	2	1.07	0.5	>200	7.18
15	0.5	2	0.88 (0.65*)	0.5 (0.5*)	428	7.95
FK518	1	16	1.51	1	46.9	Non-basic

[a] Class A TEM β-lactamase-induced clinical isolate.
[b] Class C AmpC(ld) β-lactamase-induced clinical isolate.
[c] Fifty-four clinical isolates (*196 clinical isolates).
[d] Convulsion-inducing effect: ED_{50} (μg/head, mouse intracerebroventricular injection).

convulsion-inducing effect (ED_{50}; 4.69 μg/head, mouse intracerebroventricular injection). Next we altered this guanidine part to an amino group to attempt to weaken the convulsion-inducing effect by controlling basicity. As we predicted, the convulsion effect of **12** was relatively low, but not as low as that of CZOP. Interestingly, the activity of **11** against the Class C β-lactamase-producing strain remained strong (MIC: 1 μg/mL). Further study altering the length of the alkyl chain at the 4-position of the pyrazolium ring showed that an aminopropyl group (**12**) had the best profile against this strain (data not shown). A 3D-structure based study and enzyme inhibitory study suggested steric effects were important for stability to Class C (AmpC) β-lactamase.[12]

Next, we investigated the effect of this side chain on anti-pseudomonal activity and convulsion-inducing effect. Sterically-restricted derivatives **13-15** were synthesized and evaluated. Compound **15** was synthesized as shown in Scheme 2. While antibacterial activities of these compounds were maintained, a significant reduction in the convulsion-inducing effect was observed (Table 4). Compound **13** having the *E*-form olefin showed a lower convulsion-inducing effect than non-conformationally restricted derivative **12**. The glycine analog (**14**) also showed a weak convulsion-inducing effect, presumably due the further reduced pK_a of the amino group (pK_a = 7.18), but this derivative also showed the weakest mean MIC of the derivatives prepared. The optimal compound obtained was compound **15** (FR264205). This analog showed the best balance of MIC against the Class C β-lactamase-producing strain, mean MIC against 54 clinically isolated strains, and the weakest convulsion-inducing effect in mice, showing significantly weaker activity than the marketed cephems CAZ and CZOP.

In summary, the synthesis and anti-pseudomonal activities of a series of novel cephalosporin derivatives have been explored based on rationally improving activity by increasing outer-membrane permeability by introduction of various basic amino substituents to the 3-position substituent. Introduction of amino groups to the 4-position of a 3-amino-2-methylpyrazole cephalosporin resulted in improved MIC values against Class C β-lactamase-inducing *P. aeruginosa* strains. Furthermore, while convulsion-inducing activity (due to CNS effects) was comparable to the marketed cephems, we required an analog with significantly reduced potential, and discovered that conformational restriction of the 4-position substituent on the pyrazolium ring reduced this potential dramatically and led to the discovery of FR264205 which is a promising derivative with excellent anti-bacterial activity suitable for further evaluation.

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