SYNTHESIS AND BIOLOGICAL PROPERTIES OF FCE 25199, A NEW ORAL PENEM

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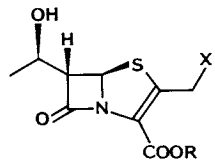
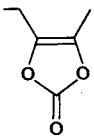
Among non-classical β-lactam antibiotics, considerable attention is being paid to the carbapenems and penems[1]. So far, the possibility of oral administration, either as such (Suntory SUN 5555[2]) or after ester-type prodrug derivatization (Farmitalia Carlo Erba FCE 22891[3]), has been a distinct advantage of the penems over the carbapenem family. New orally active penems should combine superior biological properties with production costs acceptable in a price-sensitive market. On this basis FCE 25199 (**2**), the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester of (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-methoxymethylpenem-3-carboxylic acid (**1**; FCE 24964[4]), was selected for further studies. Structurally related to the oral cephalosporin cefpodoxime[5] because of the methoxymethyl substituent, FCE 25199 features a new prodrug moiety[6], also found in lenampicillin[7] and cefcanel daloxate[8], which is claimed to release non-toxic metabolites *in vivo*[9].

Synthetic strategies which entailed an early introduction of the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group, thus obviating the temporary protection of the penem carboxyl function, were



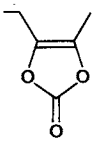
1 (FCE 24964) X = OCH_3 R = Na

2 (FCE 25199) X = OCH_3 R =



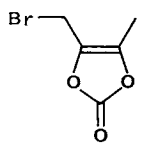
3 (FCE 22101) X = $OCONH_2$ R = Na

4 (FCE 23761) X = $OCONH_2$ R =



5 (FCE 22891) X = $OCONH_2$ R = CH_2OCOCH_3

Scheme 1.



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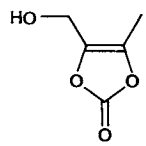

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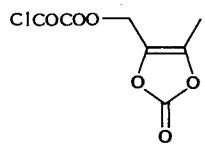

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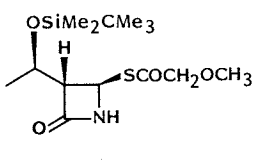

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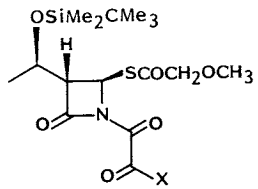
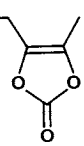
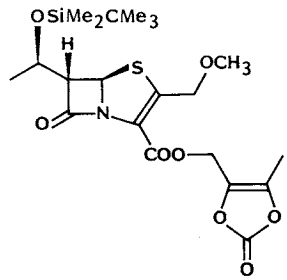


10 X = Cl
11 X = OH
12 X = -O-





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2 (FCE 25199)

carefully examined[10,11] (Scheme 1). Following an original methodology developed for FCE 22891 systhesis[12], the oxalimide chloride **10** was obtained by acylation of (3*S*,4*R*)-3-[(*R*)-1-*tert*-butyldimethylsilyloxyethyl]-4-(methoxyacetylthio)azetidin-2-one (**9**) with oxalyl chloride in the presence of triethylamine and calcium carbonate (CH_2Cl_2, 0°C, 10 minutes, quantitative), and next hydrolyzed *in situ* (water, 10 minutes) to the acid **11**. Reaction of **11** with the bromide **6** (NEt_3, CH_3CN, 40°C, 2 hours; then aqueous work-up) allowed the isolation of crude ester **12** in apparently good yield. Treatment of **12** with triethyl phosphite in refluxing xylene for two hours provided the protected penem **13**. Following customary desilylation procedure (Bu_4NF-AcOH in THF, overnight), FCE 25199 was obtained (25% yield based on **9**): MP (°C) 109~110; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, d, J=6.3 Hz), 1.88 (1H, d, J=4.8 Hz, exch. D_2O), 2.18 (3H, s), 3.41 (3H, s), 3.73 (1H, dd, J=1.6 and 6.5 Hz), 4.25 (1H, m), 4.59 (2H, ABq, J=15.7 Hz), 4.95 (2H, ABq, J=14.0 Hz), 5.59 (1H, d, J=1.6 Hz); IR ν_{max} (KBr) cm^{-1} 3450, 1820, 1780, 1735, 1710, 1580; UV λ_{max}^{EtOH} nm (ε) 324 (8,600); $[\alpha]_D^{25}$ +139° (*c* 1.0, MeOH).

The alkylation of acid **11**, and the contaminants present in the product **12** (which cannot be purified without extensive loss), were found to be critical for the yield of the overall sequence. Taking advantage of the possibility of converting the 4-bromomethyldioxole **6** to the stable carbinol **7**[13,14], which has no equivalent in conventional reagents for the synthesis of ester-type prodrugs[15], we found that the oxalimide ester **12** could be cleanly prepared by simply adding **7** and a second equivalent of triethylamine to a cold solution of **10** resulting, as above described, from reaction of **9** and oxalyl chloride. The whole sequence was now accomplished

Table 1. Antibacterial activity[a,b] of FCE 24964 in comparison with relevant orally absorbed β-lactam antibiotics[c].

Organism	MIC (μg/ml)					
	FCE	AMP	CXM	CEC	CFIX	SUN
S.a. ATCC 13709	0.045	0.045	0.78	0.78	6.25	0.045
S.a. 39/2 Pen+	0.09	0.39	0.78	0.78	6.25	0.09
S.e. ATCC 12228	0.09	0.78	0.39	0.78	3.12	0.09
S.p. ATCC 12384	0.022	0.022	0.09	0.09	0.022	0.022
S.f. ATCC 6057	1.56	0.78	>50	25	0.19	6.25
E.c. K12	0.39	1.56	3.12	0.39	0.19	0.19
E.c. R6K (TEM 1)	0.39	>50	3.12	0.78	0.19	0.19
E.c. RP1 (TEM 2)	0.78	>50	3.12	3.12	0.19	0.39
E.c. p453 (SHV-1)	0.39	>50	0.78	1.56	0.39	0.39
E.c. R997 (HMS-1)	0.39	>50	3.12	6.25	0.19	0.39
E.c. RGN 238 (OXA-1)	0.78	3.12	3.12	0.78	0.39	0.39
E.c. R46 (OXA-2)	0.39	25	1.56	0.39	0.19	0.39
E.c. R57b (OXA-3)	0.39	25	1.56	0.39	0.09	0.39
S.t. ATCC 14128	0.39	1.56	3.12	0.39	0.09	0.19
K.a. 1522E	0.39	12.5	1.56	1.56	0.011	0.39
K.a. 1082E cef. R	0.39	>50	>50	50	0.09	0.39
E.cl. 1321E	0.39	6.25	6.25	0.78	0.09	0.39
E.cl. P99 cef. R	0.39	>50	>50	>50	>50	0.39
P. indole+	0.78	25	25	>50	0.011	0.78
P. indole−	0.78	1.56	3.12	3.12	0.005	0.78
H.i. ISS 4938	0.39	0.39	0.78	1.56	0.045	0.78
N.m. ATCC 13077	0.011	0.045	0.19	0.78	0.022	0.045
C.p. 66	0.78	0.09	12.5	12.5	6.25	0.39
C.d. CD1	12.5	>50	50	50	>50	25
B.f. BF4	0.045	25	25	>50	50	0.022

[a] Test performed on Mueller-Hinton agar for aerobes, Wilkins Chalgren for anaerobes, with inocula of 10^4 cfu/spot.

[b] Organisms included in this Table are: *S.a.*, *Staphylococcus aureus*; *S.e.*, *Staphylococcus epidermidis*; *S.p.*, *Streptococcus pyogenes*; *S.f.*, *Streptococcus faecalis*; *E.c.*, *Escherichia coli*; *S.t. Salmonella typhi*; *K.a.*, *Klebsiella aerogenes*; *E.cl.*, *Enterobacter cloacae*; *P.*, *Proteus*; *H.i.*, *Haemophilus influenzae*; *N.m.*, *Neisseria meningitidis*; *C.p.*, *Clostridium perfringens*; *C.d.*, *Clostridium difficile*; *B.f.*, *Bacteroides fragilis*.

[c] FCE=FCE 24964; AMP=ampicillin; CXM=cefuroxime; CEC=cefaclor; CFIX=cefixime; SUN=SUN 5555.

Table 2. Bactericidal activity[a] of FCE 24964 on selected laboratory strains[b].

Organism	MBC (μg/ml)	MBC/MIC	Organism	MBC (μg/ml)	MBC/MIC
S.a. ATCC 13709	0.045	1	*K.a.* 1522E	0.39	1
S.a. 39/2	0.19	2	*K.a.* 1082E[c]	0.39	1
S.e. ATCC 12228	0.19	2	*E.cl.* 1321 E	0.78	2
S.p. ATCC 12384	0.022	1	*E.cl.* P99[c]	0.78	2
S.f. ATCC 6057	>64	>40	*P.m.* FI 7474	1.56	2
E.c. K12	0.39	1	*P.r.* ATCC 9250	3.12	2
E.c. TEM[c]	0.39	1			

[a] Aliquots from tubes that were clear after MIC determination in broth were plated on Mueller-Hinton agar and cfu counted after 18 hours incubation at 37°C. The MBC was defined as the drug concentration killing 99.9% of the starting inoculum.

[b] Organisms included in this Table are: *S.a.*, *Staphylococcus aureus*; *S.e.*, *Staphylococcus epidermidis*; *S.p.*, *Streptococcus pyogenes*; *S.f.*, *Streptococcus faecalis*; *E.c.*, *Escherichia coli*; *K.a.*, *Klebsiella aerogenes*; *E.cl.*, *Enterobacter cloacae*; *P.m.*, *Proteus mirabilis*; *P.r.*, *Proteus rettgeri*.

[c] β-Lactamase producers.

Table 3. Pharmacokinetic properties[a] and hydrolytic stability of penem prodrugs.

	FCE 25199	FCE 23761	FCE 22891
Relative bioavailability[b] (%)	74	28	56
$T_{1/2}$ β (minutes)	30	11	8
Cmax (μg/ml)	42	6.5	11
Chemical stability[c] (hours), pH 1.2	3.73	NT	1.81
pH 7.0	5.85	NT	9.12

[a] Following oral administration of 20 mg/kg equivalents of the active principles **1** or **3** in mice.

[b] Relative bioavailability (%) $= \frac{\text{AUC of prodrug (po)}}{\text{AUC of parent drug (iv)}} \times 100$.

[c] Chemical half-lives (HPLC determination) at 37°C, 0.2 M phosphate buffer, 0.2 mg/ml initial concentration, in the presence of 5% CH_3CN as solubilizing vehicle.

NT = not tested.

in appreciably higher yields (50~55%). Similar results were obtained[10,16] when the oxalimide **12** was prepared by acylation of the azetidinone **9** with the more elaborate dioxolemethyl chloro-oxalate synthon **8**†. On preparations up to kilogram scale we found the two-step/one-pot reaction of the acid chloride **10** with the alcohol **7** more practical.

The *in vitro* activity of the parent compound FCE 24964 in comparison with other orally absorbed β-lactams, including SUN 5555 (prepared in our laboratories according to a published procedure[18]), is shown in Table 1. This simple penem molecule is endowed with potent and broad antibacterial activity against Gram-positive and Gram-negative organisms (except *Pseudomonas* spp.), regardless of β-lactamase production. Also strains resistant to newer cephalosporins, such as *Enterobacter cloacae* P99 and *Klebsiella aerogenes* 1082E, are inhibited by FCE 24964. The high degree of bactericidal activity of FCE 24964 is documented in Table 2; with the exception of *Streptococcus faecalis*, the MBC/MIC ratio is constantly low (≤2). Relatively good stability to porcine renal dehydropeptidase was observed *in vitro*; under conditions previously reported[19], the calculated specifity constant (Vmax/*Km*) of the enzyme (1 μg/ml concentration) for FCE 24964, FCE 22101 and imipenem was 9.0, 21.6 and 23.0 (10^{-3} minute^{-1}), respectively.

While the oral absorption of FCE 24964 was negligible, high and prolonged plasma levels in mice and rats were observed after administration of ester prodrug formulations. Among several of them, the dioxolemethyl ester FCE 25199 was selected. Its excellence (Table 3) is in sharp contrast with results obtained from FCE 22101, whose corresponding ester FCE 23761 (**4**) was by far inferior to the

† After completion of our studies, the preparation and use of this reagent for the synthesis of a related penem was reported by Sankyo[17].

Table 4. *In vivo* activity of FCE 25199 in comparison with FCE 22891, SUN 5555 and cefixime (CFIX) in mouse systemic infections[a].

Organism[b]	Treatment[c]	ED_{50} (mg/kg)[d]			
		FCE 25199	FCE 22891	SUN 5555	CFIX
S.a.	120	1.3	1.2	10.4	71.0
S.p.	120	3.2	3.1	12.4	5.0
E.c.	30-90-360	12.5	20.5	44.0	2.1
K.p. (+)	30-90-360	16.8	16.9	19.2	0.12

[a] Inoculation by ip route, 8 CD1 female mice for each experimental group. Infecting dose = 3 LD_{50} for *K.p.* and 10 LD_{50}s for other organisms.
[b] Infecting organisms are: *S.a.*, *Staphylococcus aureus* ATCC 13709; *S.p.*, *Streptococcus pyogenes* ATCC 12384; *E.c.*, *Escherichia coli* G; *K.p.* (+), *Klebsiella pneumoniae* NIG6 (β-lactamase producer).
[c] Minutes after infection.
[d] Cumulative oral dose.

acetoxymethyl analogue FCE 22891 (**5**). The stability of the dioxole moiety towards chemical hydrolysis at gastric and intestinal pH values can be considered satisfactory. The prompt availability of the bioactive agent after oral absorption of FCE 25199 is guaranteed by the fast enzymic hydrolysis monitored in 50% mouse serum ($T_{1/2} \leq 2$ min), which was accompanied by a virtually quantitative release of FCE 24964.

Preliminary data on the efficacy of FCE 25199 in mouse systemic infections, in comparison with the two oral penems under clinical evaluation and cefixime, are presented in Table 4. In these experiments the four compounds were tested in parallel against the listed pathogens. The ED_{50}s for FCE 25199 and FCE 22891 were comparable, and always lower than those of SUN 5555. In conclusion, FCE 25199 merits further development to assess its absorption and pharmacokinetics after oral dosing in humans.

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