SYNTHESIS AND BIOLOGICAL PROPERTIES OF SODIUM (5*R*,6*S*,8*R*)-6α-HYDROXYETHYL-2-CARBAMOYLOXYMETHYL-2-PENEM-3-CARBOXYLATE (FCE 22101) AND ITS ORALLY ABSORBED ESTERS FCE 22553 AND FCE 22891

Sir:

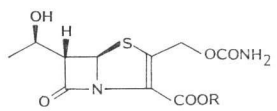
As a part of our program specifically designed to synthesize "2-CH_2X penem" variants,[1,2] distinguishable from the "2-alkylthio" analogues,[3,4] we singled out the carbamoyloxy residue as a very appealing moiety on account of the high *in vivo* metabolic stability conferred by this group to cefoxitin and cefuroxime.

We wish to describe here the synthesis and the biological properties of the new penems (**1a**,[5] **b**, **c**) bearing some unique structural features already present in thienamycin and cephamycins.

Nucleophilic displacement of the acetoxy group of **2**[4] with a suitably *O*-protected thioglycolic acid, gave **3**; mp 123~125°C, $[\alpha]_D^{20}$ +90° ($CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$); δ 0.07 (s, 6H, $Si(CH_3)_2$), 0.88, 1.11 (two s, 18H, $SiC(CH_3)_3 \times 2$), 1.23 (d, J=5.5 Hz, 3H, CH_3CH), 3.24 (dd, J=2.5 Hz, 1H, H-3), 4.15~4.20 (m, 1H, CH_3CH), 4.24 (s, 2H, $COCH_2O$), 5.24 (d, J=2 Hz, 1H, H-4), 7.30~7.70 (m, 10H, $SiPh_2$). According to WOODWARD's procedure the azetidinone-thioester **3** was then condensed with *p*-nitrobenzyl glyoxylate: chlorination of the resulting epimeric carbinolamides and subsequent reaction with triphenylphosphine afforded phosphorane **4**. Selective deprotection of the primary alcohol with Bu_4NF and subsequent WITTIG ring closure, gave the important and versatile penem **5**: ^{1}H NMR (90 MHz, $CDCl_3$); δ 0.05 (s, 6H, $Si(CH_3)_2$), 0.85 (s, 9H, $SiBu^t$), 1.25 (d, J=6 Hz, 3H, CH_3CH), 3.44 (t, J=6 Hz, 1H, CH_2OH), 3.78 (dd, J=2.4 Hz, 1H, H-6), 4.29 (dq, J=2.6 Hz, 1H, CH_3CH), 4.64 (d, J=6 Hz, 2H, CH_2OH), 5.32 (two d, J=14 Hz, 2H, $COOCH_2Ar$), 5.64 (d, J=2 Hz, 1H, H-5), 7.60~8.20 (two d, 4H, $ArNO_2$).

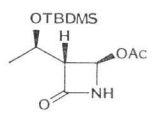
Reaction of **5** with trichloroacetylisocyanate followed by treatment with Bu_4NF allowed removal of both protecting groups to give **1d**. Final hydrogenolysis of the *p*-nitrobenzyl ester gave **1a**; ^{1}H NMR (200 MHz, D_2O); δ 1.31 (d, J=6.5 Hz, 3H, $CHCH_3$), 3.91 (dd, J=1.5, 6.0 Hz, 1H, H-6), 4.25 (dq, J=6.0, 6.5 Hz, 1H, CH_3CH), 5.02, 5.36 (two d, J=14.5 Hz, 2H, CH_2OCO), 5.66 (d, J=1.5 Hz, 1H, H-5). $[\alpha]_D^{20}$ +140° (*c* 1, H_2O). λ_{max} (H_2O) 258 (4150), 306 (6030). Chemical half-lives (spectrophotometric determination at 37.5°C): 200 hours (pH 7.4), 20 hours (pH 2.5) and 2 hours (pH 1.2). Compound **1a** was then transformed into the labile esters **1b**, **c**[6], simply by reacting the sodium salt with the corresponding acyloxymethyl halides ($(CH_3)_3CCOOCH_2Br$ and CH_3COOCH_2Br).

Compound **1a** showed an outstanding spectrum of antibiotic activity (Table 1). It was constantly more potent than cefotaxime and moxalactam (latamoxef) in inhibiting Gram-positive bacteria. The activity against Gram-negative strains was not so high as that of cefotaxime but **1a**, in contrast to the reference compounds, showed similar if not identical low MIC values on both sensitive



1a R = Na FCE 22101
1b R = $CH_2OCOC(CH_3)_3$ FCE 22553
1c R = CH_2OCOCH_3 FCE 22891
1d R = *p*-Nitrobenzyl

1a - 1d



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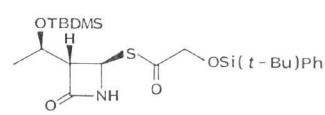

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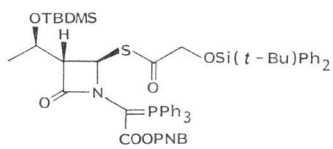

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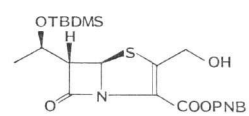

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TBDMS = *tert*-Butyldimethylsilyl

and resistant microorganisms. In addition compound **1a** exhibited remarkable activity against anaerobes. The β-lactamase resistance of **1a**, as demonstrated by the MIC test on β-lactamase producer strains, was accompanied by the ability to completely inhibit β-lactamase type **Ia** *in vitro*: complete inhibition of Nitrocefin cleavage was achieved with 0.6 μg/ml of the compound with ultrasound-disrupted cultures of *Enterobacter cloacae* P99 according to O'CALLAGHAN[7]. Fig. 1 shows the comparative plasma levels in mice when **1a** was injected intravenously and esters **1b**

Table 1. Antibacterial activity* of FCE 22101 and other β-lactam antibiotics on Gram-positive and Gram-negative bacteria as determined by the agar dilution technique.

Strain	MIC (μg/ml)		
	FCE 22101	Cefotaxime	Moxalactam
Staphylococcus aureus 209 P	0.037	1.25	0.32
S. aureus Smith ATCC 13709	0.018	0.62	0.31
S. albus FI-Pag	0.62	5	5
S. epidermidis P. S. 109	0.037	5	2.5
Streptococcus pyogenes ATCC 12384	0.15	1.25	2.5
S. faecalis ATCC 8043	2.5	>10	>10
Listeria monocytogenes ATCC 4428	0.31	1.25	>10
Escherichia coli B	0.31	0.011	0.15
E. coli B Cef R (I)	0.62	0.15	2.5
E. coli 026: B6	0.31	0.15	0.31
E. coli 026: B6 Cef R (IV)	1.25	1.25	2.5
Enterobacter cloacae 1321E	0.39	0.095	0.78
E. cloacae P99 (Ia)	0.78	10	3.12
Klebsiella aerogenes 1522E	0.78	0.095	0.19
K. aerogenes 1082 E (IVc)	0.78	6.25	0.78
K. pneumoniae ATCC 10031	0.62	0.011	0.15
Salmonella thyphimurium ATCC 14028	0.62	0.15	0.31
Proteus vulgaris ATCC 27973	1.25	0.15	0.15
P. morganii ATCC 25830	1.25	0.037	0.15
P. rettgeri ATCC 9250	1.25	0.018	0.15
Pseudomonas aeruginosa ATCC 19660	>10	10	10
Shigella flexneri ATCC 11836	0.31	0.011	0.15
Neisseria gonorrhoeae ATCC 9826	0.075	0.037	1.25
Haemophilus influenzae 716	0.18	0.022	1.25
Clostridium perfringens ATCC 13124	0.18	1.56	0.25
Bacteroides fragilis ATCC 23745	0.12	6.25	0.78

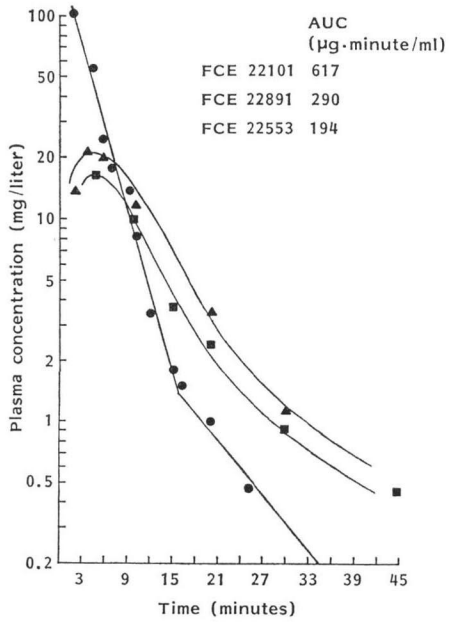
* MICs determined in Bacto Antibiotic Medium No 1, Difco (aerobes) and FTM Medium Difco (anaerobes). 5% normal rabbit blood was added to Medium No. 1 for *Streptococcus pyogenes*. Inoculum: 10^4 cells/ml. Incubation: 24 hours at 37°C.
In brackets: type of β-lactamase according to RICHMOND[5].

Table 2. *In vivo* activity of FCE 22101 and its esters FCE 22553 and FCE 22891 in comparison with cefotaxime*.

Strain	Treatment time after infection (minutes)	ED_{50} (mg/kg, cumulative dose)			
		FCE 22101**	FCE 22553***	FCE 22891***	Cefotaxime**
Staphylococcus aureus Smith ATCC 13709	120	0.48	1.8	2.0	4.0
Escherichia coli 026: B6	30, 90	16.0	40.0	26.3	18.0

* Intraperitoneal infection in mice with 3 LD_{50}; groups of 10 CD1 ♀ mice/dose.
** Subcutaneous administration.
*** Oral administration.

Fig. 1. Plasma levels of FCE 22891 (▲) and FCE 22553 (■) after oral administration, and of FCE 22101 (●) after intravenous administration at 40 mg/kg in mice.



and **1c** were administered by the oral route. The plasma concentration *vs.* time data after a single intravenous dose (40 mg/kg) of **1a** were best described according to a two compartments open model with $t_{\frac{1}{2}\alpha}=2.2$ minutes and $t_{\frac{1}{2}\beta}=7$ minutes. Area under the curve (AUC) was estimated by means of the trapezoidal rule and from the ratio of oral and intravenous AUCs, both esters resulted to be well absorbed: 47% for compound **1c** and 32% for **1b**.

The *in vivo* activity of the new penems is shown in Table 2; against *Staphylococcus aureus* infection, compound **1a** was superior to cefotaxime, while against *E. coli* its efficacy was equivalent to the reference compound. The two orally administered esters displayed also a good activity, which was well correlated with their bioavailability. Taking into account the observed plasma half-life values, the high efficacies of compounds **1a**~**1c** *in vivo* may be explained by their low protein binding (25% in mouse plasma) and their strong bactericidal activity[5]. The urinary recovery of compound **1a** in mice after intravenous administration was ≅43%. Further evaluation of these promising compounds are in progess in order to establish the clinical efficacy of this novel class of antibiotic.

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