BIOLOGICAL ACTIVITY OF
(5R,6S,8R)-6- α -HYDROXYETHYL-2-
ACETOXYMETHYL-2-PENEM-3-
CARBOXYLATE

Sir:

A few years ago we started with a program devoted to the synthesis of the so called "non classical β -lactams" which might be either potential β -lactamase inhibitors or antibiotics. More precisely we focused our attention on the synthesis of the novel 1-thia-analogue of clavulanic acid¹⁾ and on a straightforward entry into the penem family^{2,8)} starting from natural penicillins. In considering the synthetic approach, we designed in both cases a common route which involved a versatile trapping reaction on penicilanic sulphoxides⁴⁾ with the precise aim of obtain-

ing optically active compounds. Our early studies, in the penem field, were specifically oriented to the synthesis of compounds having, as a particular feature in position 2, the same functionality as the cephalosporins, *i.e.* the natural acetoxy or a derived mercaptoheterocycle group.

We have already reported the syntheses of 6-unsubstituted penems⁵⁾ and 6- α -methoxy-penems⁶⁾. The latter class, cephamycin-type, showed a disappointing antibacterial activity, presumably as a results of the inherent chemical instability.

We now wish to describe the physicochemical properties and the biological activity of 6- α -hydroxyethyl penems **1** and **2** which possess, at one and the same time the natural side chain of thienamycin and the cephalosporin functionality^{7,8)}.

Table 1. Antibacterial activity* of **1** and other β -lactam antibiotics on Gram-positive bacteria.

Strains	MIC (μ g/ml)		
	1	Cefotaxime	Latamoxef
<i>Staphylococcus aureus</i> 209 P	0.023	1.56	0.39
<i>S. aureus</i> Smith ATCC 13709	0.046	0.78	0.39
<i>S. aureus</i> FI-PV3	0.046	0.78	0.39
<i>S. aureus</i> FI-1746	0.78	0.78	0.39
<i>S. aureus</i> FI-153	0.046	0.78	0.39
<i>S. aureus</i> Tetra R ATCC 12715	0.023	0.78	0.39
<i>S. aureus</i> FI-Rifa R	0.023	1.56	0.39
<i>S. aureus</i> FI-Erithro R	0.0055	0.39	0.19
<i>S. aureus</i> FI-Spira R	0.023	0.78	0.39
<i>S. albus</i> FI-Pag.	0.39	6.25	1.56
<i>S. epidermidis</i> FK 109	0.046	6.25	1.56
<i>Streptococcus pyogenes</i> ATCC 12384	0.046	0.78	0.78
<i>S. agalactiae</i> W 182 A	0.78	50	6.25
<i>S. faecalis</i> ATCC 8043	3.12	6.25	12.5
<i>Listeria monocytogenes</i> ATCC 4428	0.39	1.56	6.25
<i>L. monocytogenes</i> 4b	0.092	3.12	1.56
<i>L. monocytogenes</i> B. T4	0.39	6.25	6.25
<i>L. monocytogenes</i> V. T4	0.39	6.25	12.5
<i>L. monocytogenes</i> C. T4	0.39	12.5	12.5
<i>L. monocytogenes</i> LR7. T4	0.39	6.25	12.5
<i>L. monocytogenes</i> LR101. T23	0.39	6.25	12.5
<i>L. monocytogenes</i> ISM 7332	0.39	6.25	6.25
<i>L. monocytogenes</i> ISM 7937	0.39	6.25	6.25
<i>Clostridium perfringens</i> ATCC 13124	0.19	1.56	0.19

* 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.

Careful hydrolysis of acetonide of **1**⁽⁸⁾ (0.1 N NaOH, acetonitrile - water, 0°C, under N₂) and purification by reverse-phase column chromatography (water as eluant) afforded the pure

(8R)-hydroxyethyl penem **1** (*threo* isomer): $[\alpha]_D^{20} +110^\circ$ (c 0.1, H₂O); PMR (80 MHz, D₂O) 1.31 (d, $J=6.5$ Hz, 3 H, CH₃CH), 2.14 (s, 3 H, OCOCH₃), 3.93 (dd, $J=1.4$ Hz, 5.8 Hz, 1 H, H-6), 4.26 (m, 1 H, CH₃CH), 5.10 and 5.46 (two d, $J=14.4$ Hz, 2 H, CH₂OCO), 5.68 (d, $J=1.4$ Hz, 1 H, H-5). IR (KBr) 3420, 1770, 1750, 1610 cm⁻¹; λ_{max} (H₂O) 258 (ϵ 4,630), 308 (6,870) nm; chemical half life 100 hours (pH 7.4, phosphate buffer, 37.5°C).

Table 2. Antibacterial activity* of **1** and other β -lactam antibiotics on Gram-negative bacteria.

Strains	MIC (μ g/ml)		
	1	Cefotaxime	Latamoxef
<i>Escherichia coli</i> B	0.39	0.011	0.19
<i>E. coli</i> B Cef R (I)	0.39	0.19	3.12
<i>E. coli</i> O26: B6	0.39	0.78	0.39
<i>E. coli</i> O26: B6 Cef R (IV)	3.12	1.56	3.12
<i>E. coli</i> K 12	0.39	0.011	0.092
<i>E. coli</i> R6K (III, TEM 1)	0.39	0.023	0.19
<i>E. coli</i> R 997 (III, HMS 1)	0.39	0.045	0.19
<i>E. coli</i> RP1 (III, TEM 2)	0.19	0.023	0.092
<i>E. coli</i> RGN 238 (V, OXA 1)	0.39	0.046	0.19
<i>E. coli</i> R46 (V, OXA 2)	0.39	0.023	0.19
<i>Enterobacter cloacae</i> P99 (I a)	3.12	>50	25
<i>E. cloacae</i> 214 (I a)	6.25	>50	>50
<i>Klebsiella aerogenes</i> 1082 E (IV c)	0.78	3.12	0.39
<i>K. pneumoniae</i> ATCC 10031	0.39	0.0055	0.092
<i>Salmonella abortusovine</i> ATCC 9842	0.092	0.011	0.046
<i>S. typhimurium</i> ATCC 14028	0.39	0.046	0.19
<i>Proteus vulgaris</i> ATCC 27973	0.39	0.046	0.19
<i>P. morganii</i> ATCC 25830	0.78	0.011	0.092
<i>P. rettgeri</i> ATCC 9250	0.78	0.011	0.19
<i>Pseudomonas aeruginosa</i> FI	0.78	0.092	0.19
<i>P. aeruginosa</i> ATCC 2598	>50	25	25
<i>P. aeruginosa</i> ATCC 19660	>50	12.5	12.5
<i>Shigella flexneri</i> ATCC 11836	0.39	0.023	0.39
<i>Neisseria gonorrhoeae</i> ATCC 9826	0.092	0.092	0.092
<i>N. gonorrhoeae</i> 1145	0.62	0.009	0.019
<i>N. meningitidis</i> ATCC 13077	0.62	0.009	0.019
<i>N. meningitidis</i> ATCC 13090	0.009	0.009	0.009
<i>Haemophilus influenzae</i> ISM-75/19	50	25	>50
<i>H. influenzae</i> 716	0.19	0.009	0.046
<i>Bacteroides fragilis</i> ATCC 23745	0.19	6.25	0.39
<i>B. fragilis</i> 61 R	0.62	10.5	0.78
<i>Bacteroides</i> sp. 57 R	0.62	0.15	1.56

In brackets: type of β -lactamase according to RICHMOND.

* MICs determined in Bacto Antibiotic Medium No. 1, Difco (aerobes) and FTM Medium Difco (anaerobes). Inoculum: 10⁴ cells/ml incubation: 24 hours at 37°C.

Analogously the (8*S*)-hydroxyethyl penem **2** (*erythro* isomer), was obtained: PMR (80 MHz, D₂O) 1.34 (d, *J*=6.5 Hz, 3H, CH₃CH), 2.14 (s, 3 H, OCOCH₃), 4.01 (m, 1 H, H-6), 4.22 (m, 1 H, CH₂-CH), 5.10 and 5.44 (two d, *J*=14.0 Hz, 2 H, CH₂OCO), 5.63 (d, *J*=1.0 Hz, 1 H, H-5); chemical half life 200 hours (pH 7.4, phosphate buffer, 37.5°C).

The *in vitro* antibacterial spectrum of **1**, determined by the agar dilution technique, is shown in Tables 1 and 2. The compound is highly active and its activity is not, or very slightly, affected by various types of β -lactamase. Moreover, no inoculum size effect could be detected on *Staphylococcus aureus* and *Escherichia coli* either β -lactamase producers or non-producers with inocula ranging from 10⁸ to 10⁷ cells/ml.

In comparison with latamoxef and cefotaxime, compound **1** is a more potent inhibitor of Gram-positive bacteria and shows a more uniform level of activity against Gram-positive strains which are generally inhibited at concentrations below 1 μ g/ml (Tables 1 and 2). *Haemophilus* and *Pseudomonas* strains more or less resistant to other widely used β -lactams such as ampicillin and cefazolin are not inhibited by **1**, whereas strains sensitive to the said antibiotics are also susceptible to **1**. Anaerobic bacteria are highly susceptible to **1**, even when rather resistant to cefotaxime.

The β -lactamase resistance of **1**, as demonstrated by the MIC test on β -lactamase producer strains, is accompanied by the ability to completely inhibit β -lactamase type Ia *in vitro*; complete inhibition of Nitrocefin cleavage was achieved with 0.3~0.6 μ g/ml of compound **1** with ultrasound disrupted cultures of *Enterobacter cloacae* P 99, according to O'CALLAGHAN⁽²⁾

The morphological modifications induced by **1** on *E. coli* cells consisted essentially of spheroplasts formation, without induction of filaments over the range of concentrations tested (1~4 $\times$ MICs). This finding could be consistent with a preferential binding to type 1 penicillin binding protein.

The high antibacterial activity observed *in vitro* for **1** is confirmed by the good therapeutic efficacy shown in experimental infections in mice (Table 3). Finally, the *erythro*-isomer **2** showed a similar profile of biological activity but the potency was 10~20 times lower than that displayed by **1**, in agreement with published data concerning activity as well as chemical half-life of related penem compounds.⁽²⁾

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Table 3. *In vivo* activity of **1** in comparison with cefotaxime. Experimental infections in mice.*

Infections	Treatments (minutes after infection)	ED ₅₀ (mg/kg, cumulative dose)	
		1	Cefotaxime
<i>S. aureus</i> Smith ATCC 13709	120	0.3	2.5
<i>S. pyogenes</i> ATCC 12384	120	0.95	0.63
<i>S. typhimurium</i> ATCC 3491	30, 60, 90	5.2	3.1
<i>E. coli</i> O26: B6	30, 90	34.4	27.3
	20, 40, 60, 80	37.4	20.6
<i>E. coli</i> O26: B6 Cef R	30, 90	21.1	80
<i>S. flexneri</i> FI	30, 60, 90	6.2	6.1

* Intraperitoneal infection in mice with 3 LD₅₀. Groups of 10 CD1 ♀♂ mice/dose; subcutaneous treatment.

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