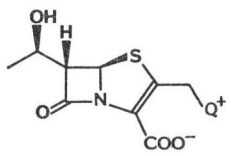
2-(QUATERNARY AMMONIO)-METHYL PENEMS

Sir:

With a view to duplicating in the penem area the enhancement of antibacterial activity that in cephalosporins is commonly associated with the inductive effect and the leaving group ability of the 3′-substituent[1], we have long been focusing our interest in 6-hydroxyethyl penems bearing a 2-CH_2X side chain. Recently we showed that an acetoxy[2], a carbamoyloxy[3] and a heterocyclylthio[4] substituent play a definite role[5] in contributing to good antibacterial performance. Now we wish to report our preliminary results on the new class of 2-(quaternary ammonio)methyl penems **1**[6].

The ammonium groups chosen for a representative set of products included pyridinium (**a**~**g**), trialkylammonium (**h**), anilinium (**i**), cycloalkylammonium (**j**~**n**) and quinuclidinium (**o**, **p**) derivatives; a number of these constitute the 3′-substituent of well-known cephalosporins, such as cephaloridine, ceftazidime (**a**), cefsulodin (**b**), cefpirome (**f**), and Bristol-Myers BMY 28142 (**j**).

The synthetic procedure of choice made use of the 2-hydroxymethylpenem **2a**, whose activation as a mesylate was sufficient for the introduction of heterocyclyl thiols[4]. The aromatic and tertiary amines considered here did not react under the mild conditions dictated by the instability of the mesylate; however, triflation (trifluoromethanesulfonic anhydride, CH_2Cl_2, −40°C) in the presence of ≥2 mol equiv of the amine Q (acting both as an acid acceptor and an *in situ* nucleophile) did yield the intermediate ammonium salts **4a**, except when Q is isoniconitamide. Compound **1b** was obtained by an alternative approach, stemming from the observation that 3-bromomethyl-2-thiacephems, differently from 2-bromomethylpenems, are easily isolatable chemical entities[7]. The bromide **5b** underwent a slow but clean displacement with isonicotinamide (DMF, 25°C, 20 hours, 66%) affording the desired ammonium salt **6b**, which was desilylated to **6c** ($BF_3 \cdot (C_2H_5)_2O$, propylene oxide, CH_3CN, 0°C, 30 minutes) and then ring-contracted (PPh_3, acetone, 25°C, 5 minutes) into a separable mixture (3:2) of 5*R* and 5*S* penems **4c** (Q^+ = iso-



1a ~ 1p

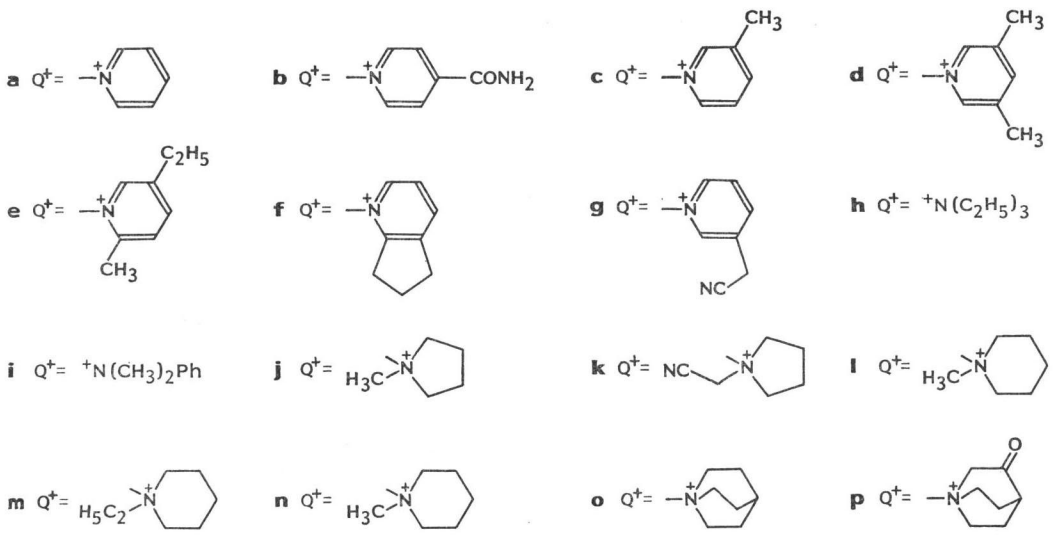


Table 1. *In vitro* antibacterial activity[a,b] of penems.

Compound	*S.a.*	*S.p.*	*E.f.*	*K.a.*	*K.a.*+	*E.c.*	*E.c.*+	*E.cl.*	*E.cl.*+	*P.*ind.+	*C.f.*	*P.a.*
1a	≤0.006	≤0.006	12.5	1.56	0.78	0.19	0.39	0.78	0.78	6.25	0.78	14.0
1b	≤0.006	≤0.006	12.5	3.12	0.78	0.78	1.09	0.78	0.78	8.8	1.56	17.7
1c	≤0.006	0.012	12.5	1.56	1.56	0.54	0.78	0.78	0.78	6.25	1.56	25
1d	≤0.006	≤0.006	12.5	1.56	1.56	0.54	1.09	1.56	1.56	25	1.56	100
1e	0.006	≤0.006	25	0.27	0.27	0.08	0.27	0.19	0.78	4.42	0.27	50
1f	≤0.006	≤0.006	12.5	0.78	0.78	0.39	0.39	0.78	1.56	4.42	0.78	35.4
1g	≤0.006	≤0.006	12.5	0.78	0.78	0.39	0.39	0.39	0.78	3.12	0.78	14
1h	≤0.006	≤0.006	100	0.19	0.1	0.024	0.39	0.1	0.19	0.39	0.1	>100
1i	0.022	0.022	50	6.25	3.12	1.1	2.2	3.12	3.12	6.25	3.12	>100
1j	≤0.006	≤0.006	12.5	0.19	0.049	0.024	0.049	0.049	0.049	1.1	0.1	35.4
1k	0.022	0.049	50	0.39	0.19	0.06	0.19	0.39	0.19	3.12	0.39	>100
1l	0.006	0.006	25	0.39	1.56	0.06	0.1	0.19	0.1	2.2	0.19	>100
1m	0.006	0.006	50	0.39	0.19	0.049	0.19	0.1	0.19	2.2	0.19	>100
1n	≤0.006	≤0.006	50	0.19	0.19	0.1	0.13	0.19	0.19	1.1	0.19	100
1o	≤0.006	≤0.006	25	1.56	1.31	0.3	0.65	0.78	0.78	3.7	1.1	50
1p	≤0.006	≤0.006	12.5	1.56	1.56	0.54	0.78	1.56	1.56	4.4	1.56	50

[a] MICs (μg/ml) were determined by the standard two-fold agar dilution method in Bacto Antibiotic Medium 1 (Difco). Spots 10^4 bacteria were automatically applied to the surface of the agar using a multipoint inoculator.

[b] Organisms included in this Table are: *S.a.*; *Staphyloccus aureus* Smith, *S.p.*; *Streptococcus pyogenes* ATCC 12384, *E.f.*; *Enterococcus faecium* ATCC 8043, *K.a.*; *Klebsiella aerogenes* 1522 E, *K.a.*+; *K. aerogenes* 1082 E (producer of β-lactamase), *E.c.*; *Escherichia coli* B and 0.26: B6 (geometric mean of two determinations), *E.c.*+; *E. coli* B β-lactamase+ and 0.26: B6 β-lactamase+ (geometric mean of the two determinations), *E.cl.*; *Enterobacter cloacae* 1321 E, *E.cl.*+; *E. cloacae* P99 (producer of β-lactamase), *P.*ind.+; *Proteus* indole+(geometric mean of two determinations), *C.f.*; *Citrobacter freundii* ATCC 8090, *P.a.*; *Pseudomonas aeruginosa* 2598 and ATCC 19660 (geometric mean of the two determinations).

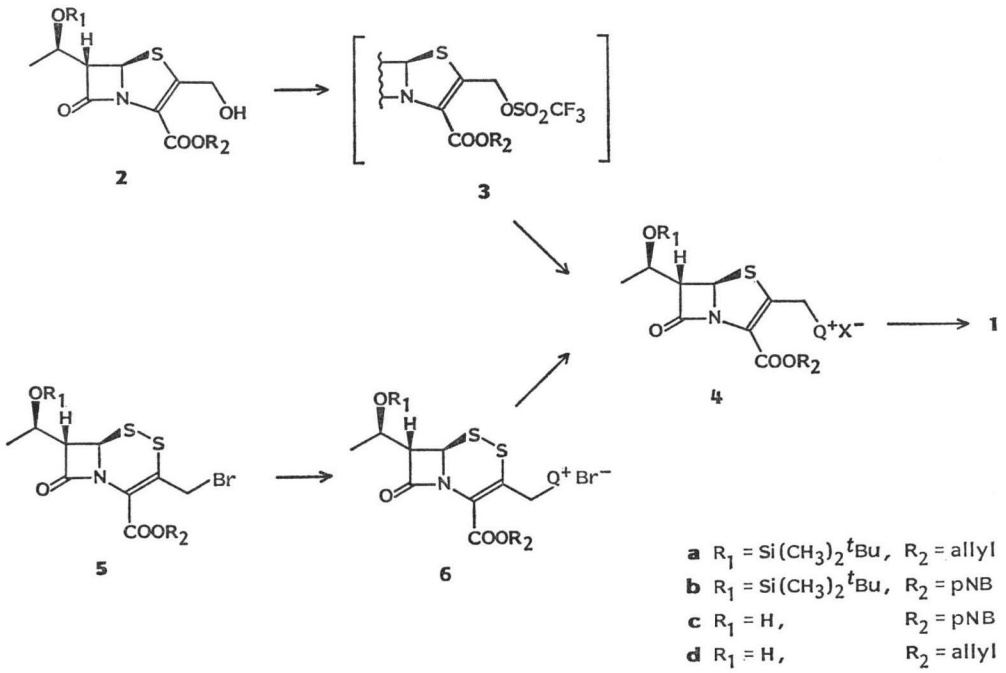


Table 2. Antibacterial activity[a] of four (quaternary ammonio)methyl penems against clinical isolates.

Microorganism	Number of strains	Compound			
		1h	**1j**	**1l**	**1n**
MRSA[b]	5	0.09	0.04	0.14	0.09
S. epidermidis	4	3.6	0.64	4.4	3.1
E. faecalis	7	3.1	0.80	4.6	4.6
E. coli β-lactamase+	5	0.045	0.045	0.27	0.19
Proteus indole+	5	0.75	0.42	3.12	1.0

[a] See footnote in Table 1. [b] Methicillin resistant *Staphylococcus aureus*.

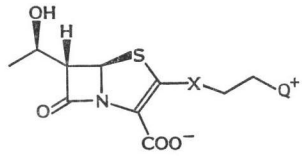
Table 3. Pharmacokinetic parameters[a] of four (quaternary ammonio)methyl penems.

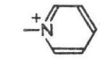
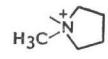
Parameter	Compound			
	1h	**1j**	**1l**	**1n**
t 1/2 α	—	6.7	—	—
t 1/2 β	5.3	7.9	4.9	5.3
AUC (μg/ml/minute)	119	119	133	124
Vd (ml/kg)	641	966	531	714
C_0 (μg/ml)	15.6	10.4	18.8	14
Pl.Cl (ml/kg/minute)	83.8	100	65.2	80.7

[a] After iv administration at 10 mg/kg in mice.

nicotinioamido, $X^-=Br^-$, 41% over the two steps). The target zwitterions **1** were obtained from intermediates **4a** after desilylation ($Bu_4NF \cdot 3H_2O$ 3 mol equiv, AcOH 10 mol equiv, THF, 25°C, overnight) and catalytic transallylation with excess acetic acid ($Pd(Ph_3)_4$, PPh_3, CH_3CN - CH_2Cl_2, 25°C, 30 minutes) or from **4c** by reductive cleavage of the pNB (*p*-nitrobenzyl) moiety (Fe, NH_4Cl, H_2O - CH_3CN, 25°C, 3 hours), and purified by reverse-phase chromatography (Merck

Table 4. Antibacterial activity[a,b] of three novel penems carrying non-displaceable quaternary ammonio groups.



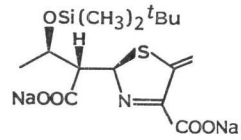
	7	8	9
X	S	CH_2	CH_2
Q^+			
S.a.	0.022	0.011	0.045
K.a.+	0.78	1.56	6.25
E.c.	0.39	0.54	2.2
E.c.+	0.54	0.78	3.12
E.cl.+	0.78	0.78	3.12
*P.*ind.+	6.25	6.25	17.7
C.f.	0.78	1.56	6.25
P.a.	12.5	35.4	50

[a,b] See footnote in Table 1.

LiChroprep RP-18, H_2O - CH_3CN). Chemical details on different combinations of R_1, R_2 and on side reactions (6, 8 dehydration, endo-exo double bond equilibration) will be given elsewhere.

This novel class of penems is characterized by a broad spectrum of antimicrobial activity, not affected by β-lactamases (Table 1). All compounds showed excellent activity against Gram-positive bacteria, with the exception of *Enterococcus faecium*, and the activity against Gram-negatives ranged from moderate to good depending on the quaternary ammonium moiety. Activity against *Pseudomonas aeruginosa* varied from slight to none. High antibacterial activity was found at superior levels in four representatives of the aliphatic and cycloaliphatic series (**1h, 1j, 1l, 1n**), which were selected for further studies (Tables 2 and 3). Among these, the *N*-methylpyrrolidinium derivative (**1j**), based on potency, broadness of spectrum (including methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* and *Enterococcus faecalis*), and pharmacokinetic properties emerged as the prime candidate.

These promising characteristics prompted us to extend our interest in quaternary ammonio-substituted penems outside the "cephalosporin-like" family **1**. Accordingly, novel penems **7**~**9** were prepared, where the inductive effect and leaving group ability of Q^+ cannot be operative (Table 4). Differing properties were found within the new series, where the *N*-methylpyrrolidinium group did not have the best profile (*e.g.*, **9** vs. **8**), suggesting that **1j** must benefit from peculiar activating factors. Model hydrolytic experiments undertaken on **1j** showed that the *N*-methylpyrrolidinium moiety, in accord with our original rationale, did behave as a leaving group; the exomethylenethiazoline **10**, reminiscent of the dihydrothiazine arising from cleavage of cephaloridine, was isolated from hydrolysis (0.1 N NaOH, 0°C, 2 hours) of **4a** (Q^+ = *N*-methylpyrrolidinium). This degradative mode, whose biological significance in determining the activity of cephalosporins is currently debated[8], has never been observed in the penem[9] and carbapenem[9,10] area so far.



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