Notes

NOVEL QUATERNARY AMMONIUM PENEMS: THE [(PYRIDINIO)METHYL]-PHENYL DERIVATIVES

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Recently we described[1] the synthesis and activity of the 2-(quaternary ammonio)methyl penems **1**, reminiscent of cephalosporins (*e.g.*, cephaloridine, ceftazidime) for the substituent at C-2. A compound, **1b**, emerged for its impressive *in vitro* potency (Table 1), but was not developed further owing to chemical stability problems and deficiencies in bactericidal activity. Since in **1** cleavage of the β-lactam ring implicates participation of the ammonium moiety as an electron sink[2] and as a leaving group[1], the question arose whether and in which direction the interposition of different spacers between the penem nucleus and the quaternary nitrogen would modify the observed antimicrobial properties.

To this end, we have examined a number of C-2 variants, which have no counterparts in the cephalosporin field. Although insertion of a saturated alkyl or thioalkyl spacer did not result in any practical advantage[1,3], a *p*-phenylene gave rise to a compound endowed with broad spectrum *in vitro* and potent *in vivo* activity, **2a**. Here we wish to report on the synthesis and properties of the group of penems **2a**~**7a**, whose peculiar [(pyridinio)methyl]phenyl moiety seems to contribute to the antimicrobial activity as a separate substructure.

Distinct procedures were required for the syn-

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

Microorganism	**1b**	**2a**	**2b**	**3a**	**4a**	**5a**	**5b**	**6a**	**7a**
S. a.	≤0.005	0.011	0.045	0.045	0.011	0.011	0.022	0.011	0.045
S. p.	≤0.005	0.011	0.005	0.011	0.011	0.005	0.011	0.005	0.01
E. f.	12.5	1.56	6.25	6.25	6.25	1.56	3.12	0.78	0.39
K. a.	0.19	0.38	0.78	1.56	0.78	0.78	0.78	1.56	0.78
K. a.+	0.045	0.38	0.78	1.56	0.78	1.56	1.56	1.56	0.78
E. c.	0.022	0.28	0.78	1.09	1.56	0.54	0.54	0.39	0.78
E. c.+	0.045	0.54	2.2	1.09	1.56	0.78	1.09	1.09	1.09
E. cl.	0.045	0.38	0.78	1.56	1.56	1.56	1.56	1.56	0.78
E. cl.+	0.045	1.56	6.25	1.56	3.12	3.12	6.25	3.12	3.12
P. ind.+	1.09	0.54	1.09	6.25	2.2	1.56	3.51	1.09	1.56
C. f.	0.1	0.19	0.78	1.56	1.56	0.78	0.78	1.56	1.56

[a] MICs (μg/ml) were determined by the standard 2-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.*, *Staphylococcus 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.

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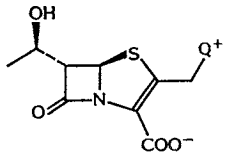

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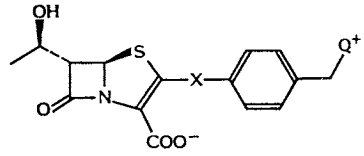
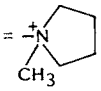
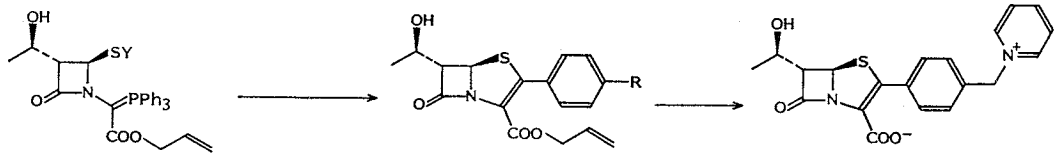
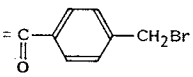
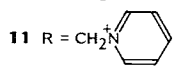


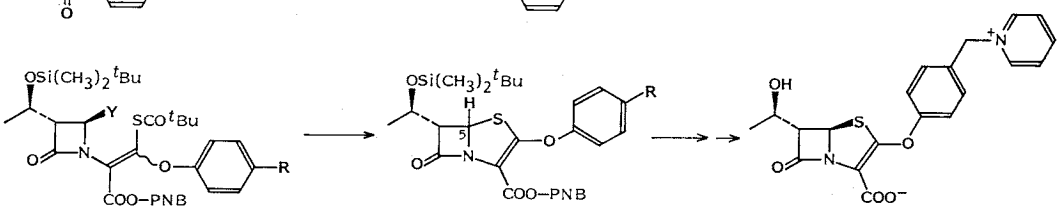
2 X = Bond **5** X = CH_2O
3 X = O **6** X = CH_2OCO
4 X = S **7** X = CH_2OCONH

a series Q^+ =  **b** series Q^+ = 

Scheme 1.



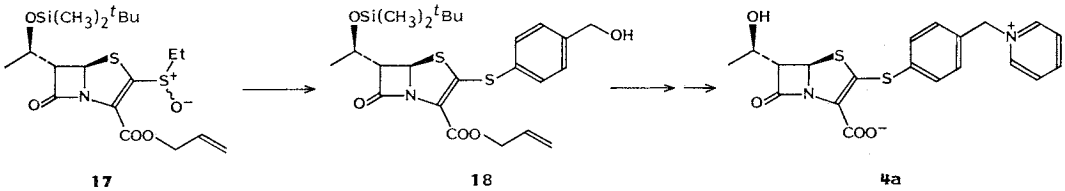
8 Y = Ag
9 Y = 
10 R = CH_2Br
11 R = 
2a



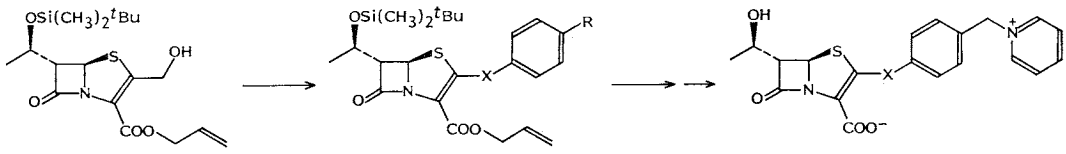
12 Y = SBu R = $CH_2OSiPh_2{}^tBu$
13 Y = Cl R = $CH_2OSiPh_2{}^tBu$
14(5*S*) R = $CH_2OSiPh_2{}^tBu$
15(5*R*) R = $CH_2OSiPh_2{}^tBu$
16(5*R*) R = CH_2OH
3a

PNB = *p*-Nitrobenzyl

Scheme 2.



17
18
4a



19
20 X = CH_2O R = CHO
21 X = CH_2OCO R = $CH_2OSiPh_2{}^tBu$
22 X = CH_2OCONH R = $CH_2OSiPh_2{}^tBu$
5a ~ **7a**

thesis of the new compounds, depending on the type of the X link. For X=bond, the bromomethylphenyl penem intermediate **10** was obtained through acylation of the silver mercaptide **8**[4] with α-bromo-*p*-toluoyl chloride in the presence of 2,6-lutidine, followed by Wittig-type ring closure (refluxing toluene) of the resulting thioester-phosphorane **9**. Displacement of the benzylic bromide with pyridine in DMF and catalytic transallylation[5] with excess acetic acid provided a straightforward access to **2a**. The route to 2-phenoxy penems involved 1,5-cyclization[6] of the chloro-thioenol arising from mild hydrolysis of the *S*-pivalate **13**, in turn obtained by condensation of the active methylene of a (4-butylthio-1-azetidinyl)acetate precursor[7] with *O*-4-(*tert*-butyldiphenylsilyloxymethyl)phenyl chlorothionoformate ($LiN(Si(CH_3)_3)_2$, THF, $-40°C$), followed by mercaptide quenching (pivaloyl chloride) and thioether chlorinolysis (Cl_2 in CH_2Cl_2, $-40°C$). Although cyclization occurred with complete inversion at C-4, the resulting (5*S*)-penem **14** underwent thermal equilibration to a separable mixture containing a major proportion (7 : 3) of the desired (5*R*)-epimer **15**. Selective unmasking of the primary hydroxyl of latter (Bu_4NF - AcOH in THF) set the stage for its activation and *in situ* displacement (triflic anhydride - pyridine, CH_2Cl_2, $-40°C$); further desilylation and ester hydrolysis (Fe - NH_4Cl)[1] afforded the target zwitterion **3a**. For the synthesis of **4a** the ethylsulfinyl penem **17** was treated with 4-(hydroxymethyl)thiophenol in the presence of diisopropylethylamine. Addition/elimination[8] resulted in intermediate **18**; therefrom introduction of the pyridinium group, desilylation and deallylation were carried out as before.

Penems **5a**~**7a** share with FCE 22101[9] the 2-hydroxymethyl precursor **19**. The ester and ether linkages were assembled through the MITSUNOBU-VOLANTE procedure[10]; however, while condensation of **19** with 4-(*tert*-butyldiphenylsilyloxymethyl)benzoic acid afforded **21** conveniently, phenols failed to react to any useful extent, unless substituted by an electron-withdrawing group. Therefore, we selected *p*-hydroxybenzaldehyde as an activated-protected synthetic equivalent of 4-(hydroxymethyl)phenol; the formyl intermediate **20** was in fact obtained in excellent yield and reduced (K-Selectride) to the corresponding carbinol. Finally, the carbamate **22** was prepared by addition of **19** to the appropriate isocyanate under 4-dimethylaminopyridine (DMAP) catalysis[11], and the carbinols derived from **20**~**22** were converted to the target pyridiniummethyl zwitterions **5a**~**7a** under the above-described conditions.

Substituting aliphatic or cycloaliphatic tertiary

Table 2. Antibacterial activity[a] of four [(pyridinio)methyl]phenyl penem derivatives against clinical isolates.

Microorganism	Number of strains	**2a**	**5a**	**6a**	**7a**
MRSA[b]	6	1.67	0.13	0.06	0.19
Enterococcus faecalis	8	0.06	0.5	0 41	0.78
Escherichia coli β-lactamase+	8	0.39	1.2	0.5	0.65
Enterobacter sp.	8	1.76	3.12	3.93	4.81
Serratia marcescens	6	3.18	12.5	16.5	12.5

[a] See footnote in Table 1.

[b] Methicillin-resistant *Staphylococcus aureus*.

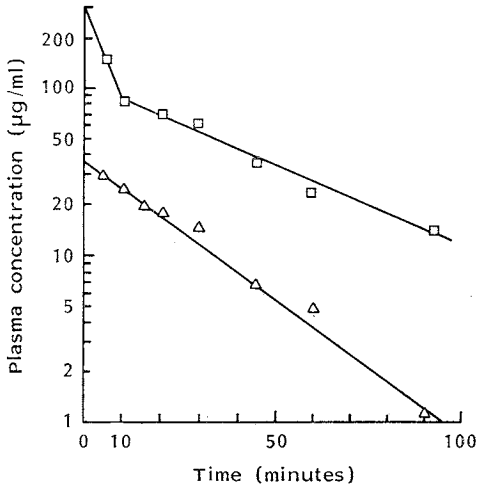
Table 3. Therapeutic efficacy of **2a** and **5a** in mouse septicemias[a].

Infection	Therapy after infection (hours)	ED_{50} (mg/kg, cumulative dose)	
		2a	**5a**
Staphylococcus aureus Smith	2	0.032	<0.032
Streptococcus pneumoniae ATCC 6303	2~24	0.96	0.5
Escherichia coli G	0.5~1.5~6	0.65	1.55
Klebsiella pneumoniae 5724	0.5~1.5~6	0.97	6.4

[a] Groups of 8–10 CDI mice were infected by intraperitoneal route and treated subcutaneously according to the reported schedule. The mortality was recorded daily and ED_{50} calculated 5 days after the infection.

Fig. 1. Plasma levels of **2a** and **5a** after intravenous administration at 20 mg/kg in the rat.

△ **2a** ($T_{1/2}$ β 22 minutes, AUC 1,061 μg·minutes/ml), □ **5a** ($T_{1/2}$ β 28 minutes, AUC 6,150 μg·minutes/ml).



amines Q for pyridine afforded the corresponding quaternary ammonium penem derivatives **2**~**7**. Two representatives of the *N*-methylpyrrolidinium series (**2b** and **5b**) are included for comparison in Table 1.

As can be seen from Tables 1 and 2, this class of penems is characterized by a broad spectrum of antimicrobial activity, including *Enterococcus faecium* but not *Pseudomonas aeruginosa*.

The derivatives **2**~**7** wherein Q^+ is pyridinium (**a** series) proved to be 2 to 4 times more active than their *N*-methylpyrrolidinium analogues (**b** series). Relative to **1b**, which *in vitro* remained the most potent quaternary ammonium penem, the novel products **2a**~**7a** were chemically more stable (data not shown) and more active *in vivo*. In particular, compound **2a** (FCE 24362) impressively emerged in the treatment of experimental infections in the mouse ($ED_{50} \leq 1$ mg/kg for both Gram-positive and Gram-negative bacteria, Table 3), while intravenous administration of **5a** in animals gave serum levels quite unusual in the class of penem antibiotics (Fig. 1). Further studies on **2a** and closely related analogues are being actively pursued.

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