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

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Recently we described¹ 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 sink² and as a leaving group²¹, 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 advantage¹, a β-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*¹ of penems.

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

* MICs (μg/ml) were determined by the standard 2-fold agar dilution method in Bacto Antibiotic Medium 1 (Difco). Spots 10⁴ bacteria were automatically applied to the surface of the agar using a multipoint inoculator.

¹ 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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Scheme 1.

8 \( Y = \text{Ag} \)

9 \( Y = \text{C} = \text{O} - \text{CH}_2\text{Br} \)

10 \( R = \text{CH}_2\text{Br} \)

11 \( R = \text{CH}_2\text{N} - \cdot \text{Br}^- \)

12 \( Y = 5\text{Bu} \)

13 \( Y = \text{Cl} \)

14(5S) \( R = \text{CH}_2\text{OSiPh}_2\text{Bu} \)

15(5R) \( R = \text{CH}_2\text{OSiPh}_2\text{Bu} \)

16(5R) \( R = \text{CH}_2\text{OH} \)

PNB = \( p\)-Nitrobenzyl

Scheme 2.

17

18

19

20 \( X = \text{CH}_2\text{O} \)

21 \( X = \text{CH}_2\text{OCO} \)

22 \( X = \text{CH}_2\text{OCONH} \)

5a - 7a
thesis of the new compounds, depending on the type of the X link. For X=bond, the bromo-
methylphenyl penem intermediate 10 was obtain-
ed through acylation of the silver mercaptide 84) with α-bromo-p-toluoyl chloride in the presence
of 2,6-lutidine, followed by Wittig-type ring
closure (refluxing toluene) of the resulting thio-
ester-phosphorane 9. Displacement of the ben-
zylic bromide with pyridine in DMF and cata-
lytic transallylation5) with excess acetic acid pro-
vided a straightforward access to 2a. The route
to 2-phenoxy penems involved l,5-cyclization6) of the chloro-thioenol arising from mild hydroly-
sis of the S-pivalate 13, in turn obtained by con-
densation of the active methylene of a (4-butyl-
thio-l-azetidinyl)acetate precursor75 with O-4-
(tert-butylidiphenylsilyloxymethyl)phenyl chloro-
thoniformate (LiN(Si(CH3)3)2, THF, -40°C),
followed by mercaptide quenching (pivaloyl
chloride) and thioether chlorinolysis (Cl2 in
CH2Cl2, -40°C). Although cyclization occurred
with complete inversion at C-4, the resulting
(5S)-penem 14 underwent thermal equilibration
to a separable mixture containing a major propor-
tion (7 : 3) of the desired (5R)-epimer 15. Selec-
tive unmasking of the primary hydroxyl of
latter (Bu4NF - AcOH in THF) set the stage for
its activation and in situ displacement (triflic
anhydride - pyridine, CH2Cl2, -40°C); further
desilylation and ester hydrolysis (Fe - NH4Cl)13)
afforded the target zwitterion 3a. For the syn-
thesis of 4a the ethylsulfinyl penem 17 was treated
with 4-(hydroxymethyl)thiophenol in the presence
of disopropylethylamine. Addition/elimina-
tion8) resulted in intermediate 18; therefrom in-
troduction of the pyridinium group, desilylation and deallylation were carried out as before.

Penems 5a~7a share with FCE 221019) the
2-hydroxymethyl precursor 19. The ester and ether
linkages were assembled through the
Mitsunobu-Volante procedure10); however,
while condensation of 19 with 4-(tert-butylidiphe-
nylsilyloxyethyl)benzoic acid afforded 21 con-
veniently, phenols failed to react to any useful
extent, unless substituted by an electron-with-
drawing group. Therefore, we selected p-hy-
droxybenzaldehyde 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 car-
bamate 22 was prepared by addition of 19 to the
appropriate isocyanate under 4-dimethylamino-
pyridine (DMAP) catalysis11), 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* of four [(pyridinio)methyl]phenyl penem derivatives against clinical isolates.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Number of strains</th>
<th>2a</th>
<th>5a</th>
<th>6a</th>
<th>7a</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSAb</td>
<td>6</td>
<td>1.67</td>
<td>0.13</td>
<td>0.06</td>
<td>0.19</td>
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<tr>
<td>Enterococcus faecalis</td>
<td>8</td>
<td>0.06</td>
<td>0.5</td>
<td>0.41</td>
<td>0.78</td>
</tr>
<tr>
<td>Escherichia coli β-lactamase+</td>
<td>8</td>
<td>0.39</td>
<td>1.2</td>
<td>0.5</td>
<td>0.65</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>8</td>
<td>1.76</td>
<td>3.12</td>
<td>3.93</td>
<td>4.81</td>
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<tr>
<td>Serratia marcescens</td>
<td>6</td>
<td>3.18</td>
<td>12.5</td>
<td>16.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* See footnote in Table 1.

b Methicillin-resistant Staphylococcus aureus.

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

<table>
<thead>
<tr>
<th>Infection</th>
<th>Therapy after infection (hours)</th>
<th>ED50 (mg/kg, cumulative dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus Smith</td>
<td>2</td>
<td>0.032</td>
</tr>
<tr>
<td>Streptococcus pneumoniae ATCC 6303</td>
<td>2~24</td>
<td>0.96</td>
</tr>
<tr>
<td>Escherichia coli G</td>
<td>0.5<del>1.5</del>6</td>
<td>0.65</td>
</tr>
<tr>
<td>Klebsiella pneumoniae 5724</td>
<td>0.5<del>1.5</del>6</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* 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 ED50 calculated 5 days after the infection.
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\textsubscript{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.

References