SYNTHESIS AND IMMUNOSTIMULATING ACTIVITY OF FK-156 ANALOGUES: FATTY ACID DERIVATIVES OF N-[N'-\(\gamma\)-D-GLUTAMYL]-L-LYSYL]-D-ALANINE

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The fatty acid derivatives 3a,b of bacterial cell-wall peptidoglycan peptides related to FK-156 (1) were synthesized and their immunostimulating activities were examined. The new compounds 3a,b showed significant antiinfectious potencies comparable to that of 1 and 3b especially exhibited a potent tumor-suppressive property lacking in 1. 

KEYWORDS — bacterial cell-wall peptidoglycan peptides; fatty acids; immunostimulating activities; antiinfection; tumor-suppression

Current interest in a unique immunostimulating property displayed by bacterial cell-wall peptidoglycan derivatives has stimulated considerable study of the chemistry of this group of natural products. In connection with a major program on FK-156 (1), a recently discovered immunostimulating microbial metabolite, we previously reported the synthesis and RES-stimulating property of its analogue 2 related to the Gram-negative bacteria peptidoglycan peptides. In continuing to explore structural modifications which would lead to either retention or enhancement of the biological potencies, we were interested in determining the effect of

\[
\begin{align*}
\text{OH} & \quad \text{CH}_3 \quad \text{D} \\
\text{CH}_3\text{CHO} & \quad \text{CO}\text{HNCHCOH} \\
\text{CH}_3\text{CHO} & \quad \text{COHNCH}_2\text{COOH} \\
\text{D} & \quad \text{L} \\
\left(\text{CH}_2\right)_2\text{COHNCHCOHNCH}_2\text{COOH} & \quad \text{L} \\
\left(\text{CH}_2\right)_3\text{COHNCHCOHNCH}_2\text{COOH} & \quad \text{D} \\
\text{H}_2\text{NCHCOOH} & \quad \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\left(\text{CH}_2\right)_5\text{COHNCHCOOH} & \quad \text{L} \\
\left(\text{CH}_2\right)_2\text{COHNCHCOHNCHCOOH} & \quad \text{L} \\
\left(\text{CH}_2\right)_3\text{COHNCHCOHNCH}_2\text{COOH} & \quad \text{D} \\
\text{H}_2\text{NCHCOOH} & \quad \text{D} \\
\end{align*}
\]

\[
\begin{align*}
\text{D} & \quad \text{R-HNCHCOOH} \\
\text{L} & \quad \text{CH}_3 \\
\left(\text{CH}_2\right)_2\text{COHNCHCOHNCHCOOH} & \quad \text{L} \\
\left(\text{CH}_2\right)_3\text{COHNCHCOHNCH}_2\text{COOH} & \quad \text{D} \\
\text{H}_2\text{NCHCOOH} & \quad \text{D} \\
\text{R} & \quad \text{NH}_2 \\
\end{align*}
\]

3a \quad R = \text{CH}_3(\text{CH}_2)_6\text{CO} \\
3b \quad R = \text{CH}_3(\text{CH}_2)_8\text{CO}
replacement of the meso-2,2'-diaminopimelic acid residue in 2 with L-Lys, 4) because the latter is a more common diamino acid especially in Gram-positive bacteria cellwall. 5) Here we report the synthesis of compounds 3a,b of this L-Lys series and their biological activity. Both proved to have significant protective effects against bacterial infection and 3b especially showed a potent tumor-suppressive activity not found in 1.

The new compounds were prepared as outlined in Chart 1. L-Lys(Z)-NCA (4) [mp 98-99℃ (lit. 6) 100℃)], prepared from Z-L-Lys(Z) 7) in 88% yield (PCl₅/CH₂Cl₂, 0℃ + reflux, 1 h), was allowed to react with D-Ala (2 equiv/McCN-H₂O, pH 10-11 with Na₂CO₃, 0℃, 1 h) to give, after purification by a HP-20 chromatography (MeOH-H₂O), H-L-Lys(Z)-D-AlaOH (5) [mp >250℃, [α]D +32.5°(c=0.2, AcOH), Rf 0.45(A) 8)] in 88% yield. Reaction of 5 with caprylund D-Glu(OH)OBzl (6a) 9) via the active ester procedure using N-hydroxysuccinimide (Et₃N/CH₂Cl₂, room temperature, 15 h) 10) gave, in 81% yield, the condensation product 7a [mp 150-152℃, [α]D -9.0°(c=0.2, AcOH)]. This was finally deprotected by hydrolysis (10% Pd-C/AcOH) to afford 3a [mp -210℃(dec.), [α]D +41.7°(c=0.2, AcOH), Rf 0.33(A), 0.69(B). Amino acid ratio of the acid hydrolysate: Glu, 1.04; Ala, 1.00; Lys, 1.09. Anal. Calcld for C₃₂H₄₆N₄O₇·2H₂O: C, 51.95; H, 8.71; N, 11.01. Found: C, 52.29, H, 8.42, N, 10.83 in 80% yield. A similar sequence of reactions from 5 and stearoyl D-Glu(OH)OBzl (6b) 9) via 7b 10) [mp 140℃, [α]D -7.7°(c=0.2, AcOH), 80% yield] yielded 3b [mp -210℃

![Chart 1](image)

(10). [α]D -11.1°(c=0.2, AcOH), Rf 0.33(A), 0.70(B). Amino acid ratio of the acid hydrolysate: Glu, 1.08; Ala, 1.00; Lys, 1.01. Anal. Calcld for C₃₂H₄₆N₄O₇·2H₂O: C, 59.23; H, 9.94; N, 8.64. Found: C, 59.60; H, 9.64; N, 8.72. 87% yield].

Compounds 3a,b and the reference compound 1 were evaluated for their ability to protect against bacterial infection and to suppress tumor growth. Table 1 shows
Table 1. Protective Effect against E. coli 22 Infection in ICR Mice (Male)\(^a\)

<table>
<thead>
<tr>
<th>Compd</th>
<th>Dose mg/Kg</th>
<th>Survival (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>controls</td>
<td>-</td>
<td>2/10</td>
</tr>
<tr>
<td>l</td>
<td>0.1</td>
<td>8/10</td>
</tr>
<tr>
<td>1</td>
<td>9/10</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>0.1</td>
<td>6/10</td>
</tr>
<tr>
<td>1</td>
<td>7/10</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>0.1</td>
<td>7/10</td>
</tr>
<tr>
<td>1</td>
<td>9/10</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Compounds were administered to mice \(i.p\) on the 4th day before challenging E. coli 22 (9 \(\times\) 10\(^7\)) by the same route. Results were obtained on the 3rd day after the bacterial challenge.

\(^b\) Number of survivors/number of mice tested.

Table 2. Suppression Effect of Meth-A Fibrosarcoma in BALB/c Mice (Female)\(^a\)

<table>
<thead>
<tr>
<th>Compd</th>
<th>Dose (\mu g/site)</th>
<th>Suppression (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>l</td>
<td>0</td>
<td>0/10</td>
</tr>
<tr>
<td>100</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>0</td>
<td>0/8</td>
</tr>
<tr>
<td>100</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>0</td>
<td>1/10</td>
</tr>
<tr>
<td>1</td>
<td>8/10</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) A mixture of Meth-A (1 \(\times\) 10\(^5\) cells) and compounds dissolved (1 and 3a) or suspended (3b) in a 0.5% solution of methylcellulose in saline was inoculated intradermally into mice. Results were obtained on the 28th day after the tumor inoculation.

\(^b\) Number of tumor-free mice/number of mice tested.

the results of an experiment on the anti-infectious effect in ICR mice against Escherichia coli 22. Compound \(3a\) showed a significant protective effect at both 0.1 mg/kg and 1 mg/kg doses, though slightly less than l, while \(3b\) showed an activity comparable to l at both doses. Although no comparison was made at this time between the new compounds and \(2\), the above data reveal that L-Lys can satisfactorily replace \(\text{meso-2,2'-diaminopimelic acid}\) in stimulating the antibacterial resistance. Compound \(3b\) is of further great interest, because it exhibited a potent tumor-suppressive activity as can be seen in Table 2. In fact, when Meth-A fibrosarcoma in BALB/c mice was used, \(3b\) was fairly effective in suppressing the tumor growth, while l and \(3a\) were entirely inactive. Note that the tumor-suppression activity was conferred by introduction of the higher fatty acid residue. This is in fair agreement with our earlier findings in the case of \(N^2-(\gamma-D\text{-glutamyl})-\text{meso-2,2'-diaminopimelic acid}\), whose higher fatty acid derivative also displayed similar antitumor activity.\(^{11}\)

This new series of compounds, especially \(3b\), should be evaluated further for their anti-infectious and antitumor potential.

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REFERENCES AND NOTES


4) Abbreviations used here for amino acids are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: J. Biol. Chem., 241, 2491 (1966); ibid., 242, 555 (1967).


8) Analytical TLC was performed with silica gel 60-F254 (E. Merck AG) using the following solvent systems: A, n-BuOH-AcOEt-H2O (5 : 2 : 3); B, n-PrOH-H2O (3 : 2).

9) Preparation of 6a,b was described in our preceding paper.11

10) The coupling reactions for obtaining 7a,b were carried out using the isolated N-hydroxysuccinimide esters of 6a,b, which were prepared by the usual DCC method: caprylyl D-Glu(OSu)OBzl, mp 67-70°C; stearoyl D-Glu(OSu)OBzl, mp 92-95°C.


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