Communications to the Editor

[Chem. Pharm. Bull. 30(8)3065—3068(1982)]

SYNTHESIS AND IMMUNOSTIMULATING ACTIVITY OF FK-156 ANALOGUES: FATTY ACID DERIVATIVES OF *N*-[N^{α}-(γ-D-GLUTAMYL)-L-LYSYL]-D-ALANINE

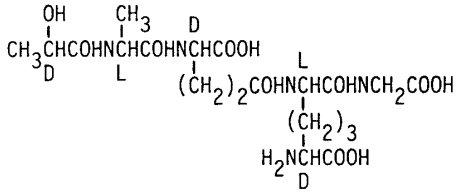
Yoshihiko Kitaura, Hidekazu Takeno, Satoshi Okada, Osamu Nakaguchi, Keiji Hemmi, Yasuhiro Mine, Joh Mori, and Masashi Hashimoto*

Central Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.
2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

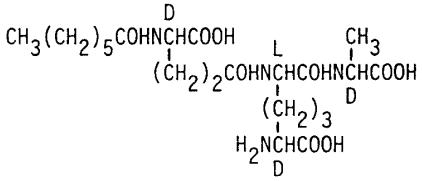
The fatty acid derivatives 3a,b of bacterial cell-wall peptidoglycan peptides related to FK-156 (1) were synthesized and their immunostimulating activities were examined. The new compounds 3a,b showed significant antiinfectious potencies comparable to that of 1 and 3b especially exhibited a potent tumor-suppressive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

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

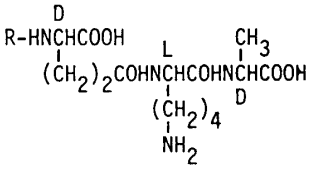
Current interest in a unique immunostimulating property displayed by bacterial cell-wall peptidoglycan derivatives has stimulated considerable study of the chemistry of this group of natural products.[1] In connection with a major program on FK-156 (1), a recently discovered immunostimulating microbial metabolite,[2] we previously reported the synthesis and RES-stimulating property of its analogue 2 related to the Gram-negative bacteria peptidoglycan peptides.[3] 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



1



2



3a R = $CH_3(CH_2)_6CO$
b R = $CH_3(CH_2)_{16}CO$

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 cell-wall.[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°C (lit.[6] 100°C)], prepared from Z-L-Lys(Z)[7] in 88% yield (PCl_5/CH_2Cl_2, 0°C → reflux, 1 h), was allowed to react with D-Ala (2 equiv/MeCN-H_2O, pH 10-11 with Na_2CO_3, 0°C, 1 h) to give, after purification by a HP-20 chromatography (MeOH-H_2O), H-L-Lys(Z)-D-AlaOH (5) [mp >250°C, $[\alpha]_D$ +32.5°(c=0.2, AcOH), R_f 0.45(A)[8]] in 88% yield. Reaction of 5 with caprylyl D-Glu(OH)OBzl (6a)[9] *via* the active ester procedure using *N*-hydroxysuccinimide (Et_3N/CH_2Cl_2, room temperature, 15 h)[10] gave, in 81% yield, the condensation product 7a [mp 150-152°C, $[\alpha]_D$ -9.0°(c=0.2, AcOH)]. This was finally deprotected by hydrogenolysis (10% Pd-C/AcOH) to afford 3a [mp ~210°C(dec.), $[\alpha]_D$ +41.7°(c=0.2, AcOH), R_f 0.33(A), 0.69(B). Amino acid ratio of the acid hydrolysate: Glu, 1.04; Ala, 1.00; Lys, 1.09. Anal. Calcd for $C_{22}H_{40}N_4O_7 \cdot 2H_2O$: 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°C, $[\alpha]_D$ -7.7°(c=0.2, AcOH), 80% yield] yielded 3b [mp ~210°C

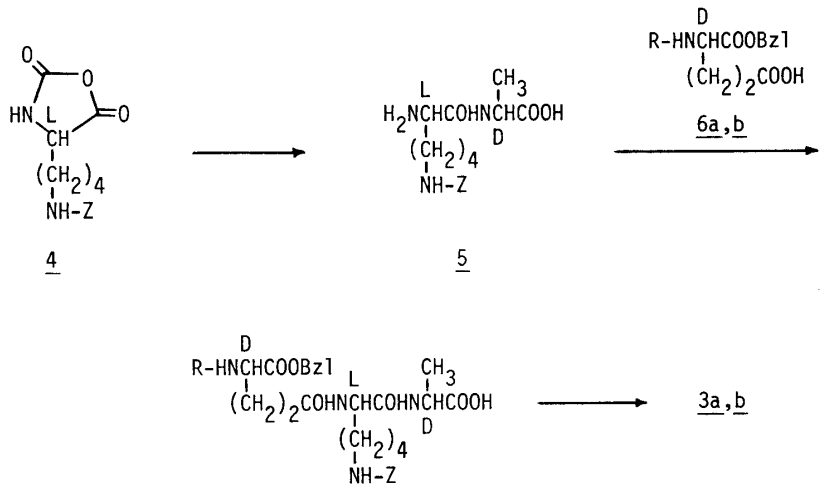


Chart 1

(dec.), $[\alpha]_D$ -11.1°(c=0.2, AcOH), R_f 0.33(A), 0.70(B). Amino acid ratio of the acid hydrolysate: Glu, 1.08; Ala, 1.00; Lys, 1.01. Anal. Calcd for $C_{32}H_{60}N_4O_7 \cdot H_2O$: 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)]

Compd	Dose mg/kg	Survival[b)]
controls	–	2/10
1	0.1	8/10
	1	9/10
3a	0.1	6/10
	1	7/10
3b	0.1	7/10
	1	9/10

a) Compounds were administered to mice *ip* on the 4th day before challenging *E. coli* 22 (5×10^7) 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)]

Compd	Dose μg/site	Suppression[b)]
1	0	0/10
	100	0/10
3a	0	0/8
	100	0/9
3b	0	1/10
	1	8/10

a) A mixture of Meth-A (1×10^5 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 antiinfectious 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 1, while 3b showed an activity comparable to 1 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 *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 1 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-(γ-D-glutamyl)-*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 antiinfectious and antitumor potential.

ACKNOWLEDGEMENT We thank S. Yonishi and Y. Morinaga for their synthetic contributions during the course of this work.

REFERENCES AND NOTES

1) See, for example, (a) E. Lederer, J. Med. Chem., 23, 819 (1980); (b) P. Leerancier and E. Lederer, Fortschr. Chem. Org. Naturstoffe, 40, 1 (1981).
2) (a) Isolation: T. Gotoh, Y. Kuroda, M. Okuhara, T. Tanaka, T. Nishiura, M. Kohsaka, H. Aoki, and H. Imanaka, Abstracts of papers, 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Il., 1981,

p. 414; (b) Synthesis: K. Hemmi, H. Takeno, S. Okada, O. Nakaguchi, Y. Kitaura, and M. Hashimoto, J. Am. Chem. Soc., 103, 7026 (1981); K. Hemmi, H. Takeno, S. Okada, O. Nakaguchi, Y. Kitaura, and M. Hashimoto, Tetrahedron Lett., 23, 693 (1982); (c) Activity: Y. Watanabe, Y. Mine, Y. Yokota, S. Tawara, M. Hashimoto, M. Nishida, S. Goto, and S. Kuwahara, in ref 2a, p. 413.

3) Y. Kitaura, H. Takeno, M. Aratani, S. Okada, S. Yonishi, K. Hemmi, O. Nakaguchi, and M. Hashimoto, Experientia, in press.

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).

5) K. H. Scheifer and O. Kandler, Bact. Rev., 36, 407 (1972).

6) M. Bergmann, L. Zerwas, and W. F. Ross, J. Biol. Chem., 111, 245 (1935).

7) R. A. Boissonnas, St. Guttmann, R. L. Huguenin, P.-A. Jaquenoud, and Ed. Sandrin, Helv. Chim. Acta, 41, 1867 (1958).

8) Analytical TLC was performed with silica gel 60-F_{254} (E. Merck AG) using the following solvent systems: A, *n*-BuOH–AcOH–H_2O (5 : 2 : 3); B, *n*-PrOH–H_2O (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.

11) Y. Kitaura, O. Nakaguchi, H. Takeno, S. Okada, S. Yonishi, K. Hemmi, J. Mori, H. Senoh, Y. Mine, and M. Hashimoto, J. Med. Chem., 25, 335 (1982).

(Received July 12, 1982)