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Short communication

The influence of the lipophilicity of 7-oxoacyl-L-alanyl-D-isoglutamines on their immunorestoration activity in vivo

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Summary — In search for immunologically active dipeptides, a series of new *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines has been synthesized where lipophilicity was varied by changing the 7-oxoacyl residue from 7-oxooctanoyl to 7-oxotetradecanoyl. The immunological properties of the compounds were examined in an in vivo immunorestoration test. It was found that lipophilicity had a significant influence on the potency of the compounds tested. Compounds between *N*-(7-oxodecanoyl)-L-alanyl-D-isoglutamine and *N*-(7-oxotetradecanoyl)-L-alanyl-D-isoglutamine showed biphasic influence on efficacy. The most active compound found was 7-oxododecanoyl-L-alanyl-D-isoglutamine, which also has an activity comparable to that of azimexone.

desmuramyldipeptide / immunomodulating activity / immunorestoration / lipophilicity

Introduction

An intensive search for useful and specific drug-stimulating immune systems for a more powerful and effective defense against pathogenic bacteria, viruses and tumor cells is carried out being on a broad spectrum of chemical compounds of varying origin. Among these, *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) (**1**, fig 1), which is the smallest immunologically active glucopeptide subunit of the bacterial cell wall, is widely explored as one of the lead compounds of natural origin. Its structure has been modified in different ways and a great deal of data on structure–activity relationships (SAR) has already been collected with the aim of obtaining MDP derivatives suitable for therapeutic use [1, 2]. The progress is limited because the receptor of MDP and the mechanism of the action are not yet known. Therefore, SAR and the conformational studies of MDP and its analogs may be a promising route to an effective immunoactive drug and more information about receptor and action mechanisms [3–5]. Of the various MDP analogs, only lipophilic MDP derivatives show effective therapeutic potential [2, 6]. Some of them (Murabutide **2**, Romurtide **3** and MTP-PE **4**; fig 1) have already been registered or are at different stages in clinical trials.

On the basis of SAR studies, it was concluded that the *N*-acetylmuramyl moiety in MDP is important but not essential for immunoactivity [7]. Its replacement with a simple fatty acyl residue (the parent dipeptide is inactive) gives the less active desmuramyldipeptides [8]. The activity of desmuramyl-

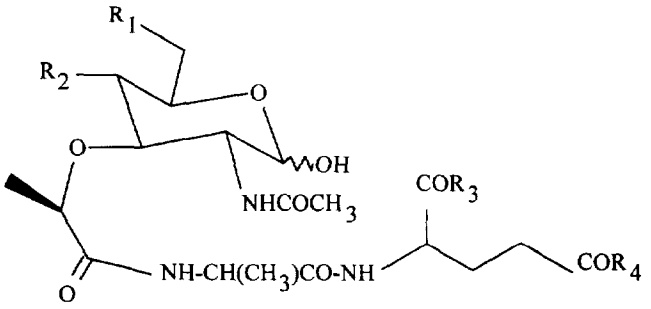

Fig. 1. Structure of MDP (**1**: $R_1 = R_2 = OH$, $R_3 = NH_2$, $R_4 = OH$), Murabutide (**2**: $R_1 = R_2 = OH$, $R_3 = n$BuO, $R_4 = NH_2$), Romurtide (**3**: $R_1 = R_2 = OH$, $R_3 = NH_2$, R_4 = L-Lys-ε-$COC_{17}H_{35}$) and MTP-PE (**4**: $R_1 = R_2 = OH$, $R_3 = NH_2$, R_4 = L-Ala-2-[1,2-dipalmitoyl-*sn*-glycero-3-(hydroxyphosphoryl-oxy)]ethylamide).

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dipeptide is restored to a greater extent if additional amino acids are added to the parent dipeptide, eg, lauroyltetrapeptide (LTP) [9]. Because of the significant reduction in the immunological activity, desmuramyldipeptides were considered as the least attractive class of compounds for further investigation. Therefore, most of the present research is concentrated on the modification of the *N*-acetylmuramyl part of MDP structure, especially by changing the lipophilicity [2, 10], by introduction of an additional *N*-acetylglucosamine group [11] or by looking for an optimal formulation [1, 2].

In our systematic search for immunoactive compounds, a number of different desmuramyldipeptides have been synthesized and tested [12, 13]. The analysis of the biological activity of the synthesized *N*-acyl-L-alanyl-D-isoglutamines [12–16] has shown that the structure of the *N*-acyl residue has an important impact on overall activity. We suppose that the *N*-acetylmuramyl group is directly responsible for the broad spectrum of MDP activities. This group acts as an 'address', directing MDP to different binding sites, while the dipeptide moiety acts as a 'messenger'. Beside the above-mentioned role of the *N*-acetylmuramyl residue, it also acts as an acyl which eliminates the positive charge of the L-alanyl amino group. Desmuramyldipeptides in which the *N*-acetylmuramyl group is replaced by a simple fatty acyl residue are practically inactive compounds probably because of the lack of or a reduction in their recognition and/or binding capacity. Analyzing the *N*-acetylmuramyl part of MDP, we propose that the carbonyl group in the *N*-acetyl part of the *N*-acetylmuramyl group might be involved in the recognition and binding to its receptor. Looking for an appropriate surrogate for the *N*-acetylmuramyl residue, we found that it could be successfully replaced by the 7-oxooctanoyl group (LK-409) [14] where all functional groups present in the sugar were eliminated except the carbonyl one. Comparison of LK-409 and MDP shows that this modification produces no important change in activity on the immune system, as shown by the tests performed [14]. The only important difference is a complete loss of the pyrogenic effect observed in LK-409 [14]. This finding confirms that one of the multiple roles of the sugar part of MDP is its contribution to pyrogenicity.

According to our present understanding, the carbonyl group on position 7 of the acyl residue essentially contributes to the activity of LK-409. Namely, the analog without the carbonyl group is completely inactive [15], whereas a more lipophilic analog, eg, 7-oxododecanoyl-L-alanyl-D-isoglutamine (LK-404), was found to be even more active than LK-409 [16]. To evaluate the influence of lipophilicity on the immunological activity, a series of desmuramyldipeptides has been prepared (fig 2), where dipeptide

$CH_3(CH_2)_nCO(CH_2)_5CO$-L-Ala-D-iGln

Compound	n
LK-409	0
LK-450	1
LK-451	2
LK-452	3
LK-404	4
LK-453	5
LK-405	6

Fig 2. LK-409 and its homologs.

(L-alanyl-D-isoglutamine) was acylated by different 7-oxo fatty acids, starting from 7-oxooctanoic to 7-oxotetradecanoic acid. The overall lipophilicity increased stepwise with increments of the methylene group while the position of the carbonyl group was unchanged.

All compounds were evaluated in an in vivo immunorestoration test measuring their general influence on the immune system.

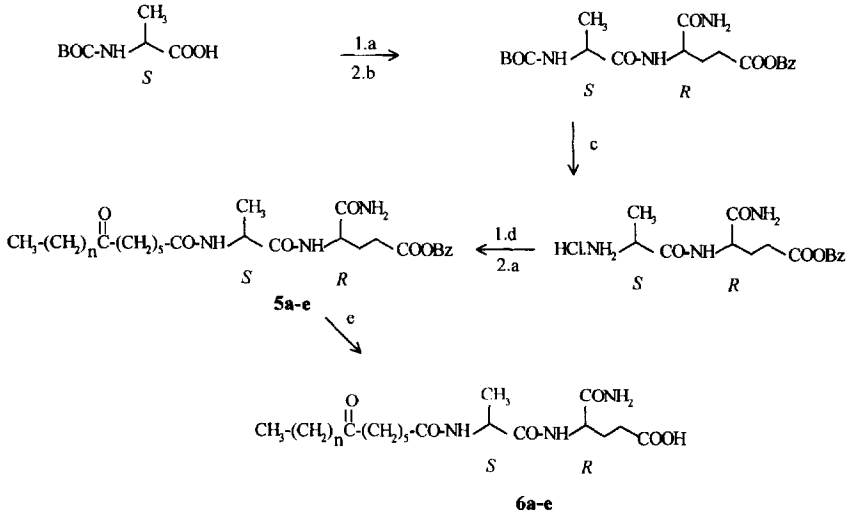
Chemistry

Table I includes all the molecules synthesized and their physical characteristics. Starting from 7-oxoalkanoic acids [17] and corresponding dipeptide fragment [18], *N*-(7-oxoacyl)-L-alanyl-D-isoglutamine benzyl esters (**5a–e**) were obtained after activation of carboxylic group with DPPA (scheme 1). The hydrogenolysis (0.5–1.5 h) of **5a–e** gave *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines (**6a–e**) in 88–96% yield. We found the DPPA-coupling method very useful; the yields were high and no racemization was observed.

Pharmacology

Immunorestoration test

In the immunorestoration test the immunological function of mice is impaired by cyclophosphamide, resulting in an increased mortality after *Candida* infection. Cyclophosphamide belongs to the classical cytotoxic immunosuppressants, which act by inhibiting the synthesis of DNA, thereby hindering the stimulus for proliferation and differentiation. These



Scheme 1. BOC: *tert*-butyloxycarbonyl; Bz: benzyl. Reagents: a) DPPA (diphenylphosphoryl azide)/Et_3N (triethylamine); b) HCl; D-iGlnBz; c) CH_3COOH/HCl; d) 7-oxoalkanoic acid; e) H_2/Pd/C.

effects result in a decreased proliferative response of all the cells involved in the immune response [19, 20]. In the cellular and humoral immune response, activated T cells and plasma cells are targeted [19]. A decrease in resistance to systemic infections in cyclophosphamide-treated mice could be caused partially by neutropenia [21]. Cyclophosphamide also decreases the functional activity of macrophages during their interaction with *Candida albicans* [22]. The immunosupressive effect of cyclophosphamide could even contribute to a decreased yeast binding to spleenic marginal zones. Namely, the binding of yeast cells to host immune cells in reticuloendothelial organs prevents their dissemination to other organs which consequently contributes to the defense against infection [23].

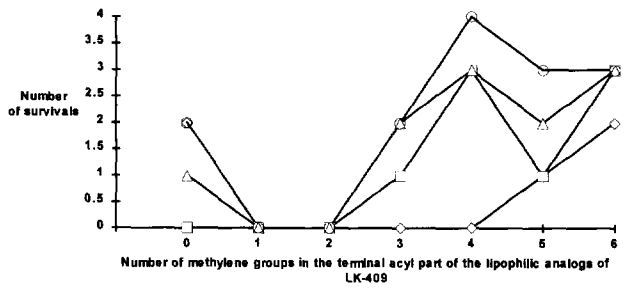
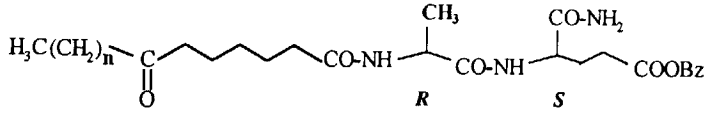


Fig. 3. Biological response of LK-409 analogs expressed as survival number of animals in immunorestoration test. ◇ 0.1 mg/kg; □ 1 mg/kg; △ 10 mg/kg; ○ 100 mg/kg.

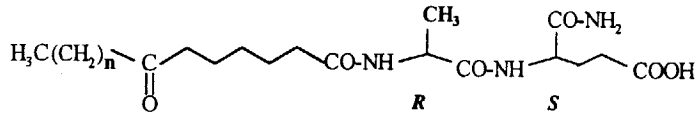
Results and discussion

As has been found previously for MDP derivatives, the introduction of a lipophilic group into the parent molecule gave MDP derivatives with a more pronounced therapeutic potential [2, 10]. The lipophilic group probably compensates for the high hydrophobic nature of MDP and consequently changes the unpleasant pharmacokinetic characteristics. The same relationship is present in our class of desmuramyldipetides where *N*-(7-oxododecanoyl)-L-alanyl-D-isoglutamine (LK-404) was found to be more potent than LK-409 [16]. A higher lipophilicity could be achieved by a simple increase in the alkyl residue bound to the carbonyl group of 7-oxo fatty acid. From our previous data we concluded that the presence and the position of the 7-oxo group in the acyl moiety is important for the immunoactivity [15]. Therefore we kept it at the same distance from the dipeptide moiety, but the lipophilicity was increased stepwise by increments of the methylene group incorporated between terminal methyl and 7-oxo group in the LK-409 molecule (fig 2). Correspondingly log *P* (*P* is the partition coefficient) also increased linearly by increments of 0.519 for each methylene group [24].

Table I. Data of benzyl esters *N*-(7-oxoacyl)-L-alanyl-D-isoglutamine **5a–e** and *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines **6a–e**.



5a–e



6a–e

Compound	*Yield (%) (solvent)*[a]	*Mp (°C)*	*Molecular formula*[b]	$[\alpha]_D^{20}$(°)	*^{1}H-NMR*[d] *(δ ppm)*
5a (n = 1)	80 (A)	150–152	$C_{24}H_{35}N_3O_6$	–8.1 (c = 0.2)	0.89 (t, 3H, J = 7.3 Hz, CH_3CH_2), 1.17 (d, 3H, J = 6.0 Hz, CH_3-Ala), 1.15–1.30 (m, 4H, $2CH_2$), 1.40–1.60 (m, 4H, $2CH_2$), 1.70–1.82 and 1.95–2.10 (2m, 1H each, β-CH_2-iGln), 2.09 (t, 2H, J = 7.5 Hz, CH_2CONH), 2.30–2.45 (m, 6H, γ-CH_2-iGln and CH_2COCH_2), 4.10–4.25 (m, 2H, α-CH-iGln, α-CH-Ala), 5.08 (s, 2H, CH_2-benzil), 7.14 and 7.33 (2s, 1H each, NH_2), 7.30–7.42 (m, 5H, phenyl), 8.08 (d, 1H, J = 8.3 Hz, NH), 8.09 (d, 1H, J = 6.3 Hz, NH)
5b (n = 2)	79 (A)	162–165	$C_{25}H_{37}N_3O_6$	–9 (c = 0.4)	0.81 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.16 (d, 3H, J = 7.08 Hz, CH_3-Ala), 1.12–1.24 (m, 2H, $CH_2CH_2CH_2$), 1.38–1.50 (m, 6H, $3CH_2$), 1.70–1.82 and 1.96–2.05 (2m, 1H each, β-CH_2-iGln), 2.08 (t, 2H, J = 7.4 Hz, CH_2CONH), 2.34 (t, 6H, J = 7.2 Hz, γ-CH_2-iGln and CH_2COCH_2), 4.10–4.22 (m, 2H, α-CH-iGln, α-CH-Ala), 5.06 (s, 2H, CH_2-benzyl), 7.13 and 7.32 (2s, 1H each, NH_2), 7.30–7.40 (m, 5H, phenyl) 8.09 (d, 1H, J = 8.4 Hz, NH), 8.10 (d, 1H, J = 6.3 Hz, NH)
5c (n = 3)	79 (A)	162–162	$C_{26}H_{39}N_3O_6$	–9.5 (c = 0.065)	0.84 (t, 3H, J = 7.3 Hz, CH_3CH_2), 1.17 (d, 3H, J = 7.02 Hz, CH_3-Ala), 1.10–1.30 (m, 4H, $2CH_2$), 1.36–1.52 (m, 6H, $3CH_2$), 1.68–1.82 and 1.98–2.05 (2m, 1H each, β-CH_2-iGln), 2.08 (t, 2H, J = 7.4 Hz, CH_2CONH), 2.30–2.55 (m, 6H, γ-CH_2-iGln and CH_2COCH_2), 4.10–4.25 (m, 2H, α-CH-iGln, α-CH-Ala), 5.08 (s, 2H, CH_2-benzyl), 7.14 and 7.32 (2s, 1H each, NH_2), 7.30–7.40 (m, 5H, phenyl), 8.05 (d, 1H, J = 8.4 Hz, NH), 8.06 (d, 1H, J = 6.3 Hz, NH)
5d (n = 5)	91 (A)	140–143	$C_{28}H_{43}N_3O_6$	–6.0 (c = 0.07)	0.84 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.17 (d, 3H, J = 6.9 Hz, CH_3-Ala), 1.10–1.30 (m, 8H, $4CH_2$), 1.35–1.55 (m, 6H, $3CH_2$), 1.70–1.85 and 1.95–2.10 (2m, 1H each, β-CH_2-iGln), 2.09 (t, 2H, J = 7.5 Hz, CH_2CONH), 2.30–2.45 (m, 6H, γ-CH_2-iGln and CH_2COCH_2), 4.10–4.23 (m, 2H, α-CH-iGln, α-CH-Ala), 5.08 (s, 2H, CH_2-benzyl), 7.13 and 7.32 (2s, 1H each, NH_2), 7.30–7.43 (m, 5H, phenyl), 8.07 (d, 1H, J = 8.4 Hz, NH), 8.08 (d, 1H, J = 6.3 Hz, NH)
5e (n = 6)	78 (A)	138–141	$C_{29}H_{45}N_3O_6$	–13.6 (c = 0.4)	0.83 (t, 3H, J = 6.9 Hz, CH_3CH_2), 1.16 (d, 3H, J = 7.08 Hz, CH_3-Ala), 1.10–1.30 (m, 10H, $5CH_2$), 1.35–1.50 (m, 6H, $3CH_2$), 1.68–1.82 and 1.95–2.01 (2m, 1H each, β-CH_2-iGln), 2.07 (t, 2H, J = 8.0 Hz, CH_2CONH), 2.28–2.40 (m, 6H, γ-CH_2-iGln and CH_2COCH_2), 4.10–4.20 (m, 2H, α-CH-iGln, α-CH-Ala), 5.06 (s, 2H, CH_2-benzyl), 7.10 and 7.30 (2s, 1H each, NH_2), 7.30–7.40 (m, 5H, phenyl), 8.05 (d, 1H, J = 8.2 Hz, NH), 8.07 (d, 1H, J = 6.4 Hz, NH)

Table I. *Continued*

Compound	*Yield (%) (solvent)*[a]	*Mp (°C)*	*Molecular formula*[b]	$[\alpha]_D^{20}$(°)	*^{1}H-NMR*[d] *(δ ppm)*
6a (n = 1)	88 (B)	195–197	$C_{17}H_{29}N_3O_6$	–9.5 (c = 0.2)	0.9 (t, 3H, J = 7.3 Hz, CH_3CH_2), 1.17 (d, 3H, J = 7.02 Hz, CH_3-Ala), 1.15–1.30 (m, 4H, $2CH_2$), 1.35–1.55 (m, 4H, $CH_2CH_2CH_2$), 1.60–1.80 and 1.90–2.05 (2m, 1H each, β-CH_2-iGln), 2.09 (t, 2H, J = 7.6 Hz, CH_2CONH), 2.18 (t, 2H, J = 7.19 Hz, γ–CH_2-iGln), 2.35–2.45 (m, 4H, CH_2COCH_2), 4.05–4.25 (m, 2H, α-CH-iGln, α-CH-Ala), 7.10 and 7.30 (2s, 1H each, NH_2), 8.08 (d, 2H, J = 7.3 Hz, 2NH)
6b (n = 2)	91 (A)	181–182	$C_{18}H_{31}N_3O_6$	–11.3 (c = 0.4)	0.81 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.17 (d, 3H, J = 7.07 Hz, CH_3-Ala), 1.15–1.22 (m, 2H, $CH_2CH_2CH_2$), 1.38–1.50 (m, 6H, $3CH_2$), 1.62–1.76 and 1.90–2.02 (2m, 1H each, β-CH_2-iGln), 2.08 (t, 2H, J = 7.8 Hz, CH_2CONH), 2.18 (t, 2H, J = 7.5 Hz, γ-CH_2-iGln), 2.35 (t, 4H, J = 7.2 Hz, CH_2COCH_2), 4.00–4.25 (m, 2H, α-CH-iGln, α-CH-Ala), 7.08 and 7.31 (2s, 1H each, NH_2), 8.05 (d, 1H, J = 7.6 Hz, NH), 8.07 (d, 1H, J = 5.9 Hz, NH)
6c (n = 3)	93 (B)	155–157	$C_{19}H_{33}N_3O_6$	–9.1 (c = 0.2)	0.84 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.18 (d, 3H, J = 7.2 Hz, CH_3-Ala), 1.15–1.30 (m, 6H, $6CH_2$), 1.35–1.55 (m, 4H, CH_2), 1.65–1.80 in 1.90–2.05 (2m, 1H vsak, β-CH_2-iGln), 2.10 (t, 2H, J = 7.6 Hz, CH_2CONH), 2.19 (t, 2H, J = 7.6 Hz, γ-CH_2-iGln), 2.38 (t, 2H, J = 7.3 Hz, CH_2CO), 2.39 (t, 2H, J = 7.3 Hz, CH_2CO), 4.10–4.25 (m, 2H, α-CH-iGln, α-CH-Ala), 7.10 in 7.31 (2s, 1H vsak, NH_2), 8.07 (d, 1H, J = 8.5 Hz, NH) 8.08 (d, 2H, J = 6.3 Hz, 2NH)
6d (n = 5)	86 (B)	157–160	$C_{21}H_{37}N_3O_6$	–6.2 (c = 0.2)	0.85 (t, 3H, J = 6.9 Hz CH_3CH_2), 1.18 (d, 3H, J = 6.9 Hz CH_3-Ala), 1.15–1.35 (m, 8H, $4CH_2$), 1.35–1.55 (m, 6H, $3CH_2$), 1.65–1.80 and 1.90–2.05 (2m, 1H each, β-CH_2-iGln), 2.09 (t, 2H, J = 7.6 Hz, CH_2CONH), 2.19 (t, 2H, J = 7.8 Hz, γ-CH_2-iGln), 2.37 (t, 2H, J = 7.4 Hz, CH_2CO), 2.38 (t, 2H, J = 7.3 Hz, CH_2CO), 4.10–4.25 (m, 2H, α-CH-iGln, α-CH-Ala), 7.11 and 7.35 (2s, 1H each, NH_2), 8.11 (d, 1H, J = 9.6 Hz, NH), 8.12 (d, 2H, J = 6.3 Hz, NH)
6e (n = 6)	96 (A)	154–157	$C_{22}H_{39}N_2O_6$	–9.9 (c = 0.4)	0.83 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.16 (d, 3H, J = 7.05 Hz, CH_3-Ala), 1.14–1.28 (m, 10H, CH_2), 1.36–1.50 (m, 6H, $3CH_2$), 1.62–1.76 and 1.88–2.02 (2m, 1H each, β-CH_2-iGln), 2.07 (t, 2H, J = 7.5 Hz, CH_2CONH), 2.18 (t, 2H, J = 7.8 Hz, γ-CH_2-iGln), 2.36 (t, 4H, J = 7.2 Hz, CH_2COCH_2), 4.05–4.25 (m, 2H, α-CH-iGln, α-CH-Ala), 7.08 and 7.32 (2s, 1H each, NH_2), 8.07 (d, 1H, J = 8.2 Hz, NH), 8.08 (d, 1H, J = 6.4 Hz, NH)

[a]Crystallization solvent: A: methanol/ether; B: acetone. [b]Anal C, H, N (analyses for all new compounds **5a–6e** agreed within 0.5% of the theoretical values). [c]Solvent: methanol. [d]Solvent: $(CD_3)_2SO$.

In figure 3 the biological response expressed as survival of animals in immunorestoration test is presented at four different doses as a function of increasing lipophilicity. At the dose of 0.1 mg/kg for compounds with n = 1–4 there was no biological response, but at higher doses, for compounds where n is 2–6, the biological response is clearly dependent on lipophilicity. The most active compound is LK-404 (n = 4). Further increase of the lipophilicity decreases the biological activity. The observed biphasic behavior (fig 3) shows that the biological effect is strongly dependent on the transport and partition properties of the compounds tested. We believe that the pyrogenic characteristics of MDP [1] but not LK-404 [16] originate from the *N*-acetylmuramyl part of MDP. This part is replaced by the 7-oxoacyl group, where only the carbonyl group is related to the sugar part. The lacking of hydroxyl groups and consequently the reduction of recognition and binding capacity to its receptor may reduce the activity of these desmuramyldipeptides but the higher lipophilicity on the other side contributes to a longer duration of action and more pronounced interaction with biological membranes. The biological activity of the compounds tested confirms the hypothesis that a simple moiety like the 7-oxoacyl group can successfully replace a more complex *N*-acetylmuramyl residue. Furthermore, it seems that the carbonyl group in the acyl residue might correspond to that from the *N*-acyl group of MDP. This group plays an important role in the recognition and binding process, as has been mentioned previously. Its position and its close surrounding are also important [15]. Immunorestoration tests also show that the lipophilicity of compounds where n = 1 and 2 has a different influence on the activity. The first compound in the series (n = 0, LK-409) is as active as the compound with n = 3 although it has lower log P, whereas compounds with n = 1 and 2 are totally inactive at all the doses applied. At this point of investigation the observed effect is not completely understood.

All compounds tested were compared to azimexone administered at dose of 100 mg/kg. Azimexone, an aziridine derivative, was chosen owing to its ability to protect mice pretreated with cyclophosphamide against *C albicans* infection. *C albicans* infections are a very common type of infection where the T lymphocyte population is either reduced or nonfunctional [25]. The protective action of azimexone in *C albicans* infection results from its immunomodulating activity and not from a direct antimicrobial activity [26]. Our compounds with n = 4 and 5 at the highest dose show an approximately equal or higher activity than azimexone. Because they have twice the molecular weight, they are even more potent than azimexone. Due to the absence of antifungicidal activity of our compounds (results not presented), their protective effect in immunorestoration test can be ascribed to the immunomodulating activity.

Experimental protocols

Chemistry

The quality of synthesized agents was determined as follows. The melting points were determined on a Kofler block and are uncorrected. ^{1}H-NMR spectra were measured on a 300 MHz Varian VXR-300 spectrometer with TMS as an internal standard. The elemental analyses were performed by Dept of Organic Chemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana. In the determination of specific rotation a Perkin-Elmer 241 MC polarimeter was used. The purity of the compounds was proved by HPLC on a Nucleosil 100 C-8 column (5 μm, 125 x 4 mm, Bia, Slovenia) using methanol and phosphate buffer (pH 3) in different proportions (50–90% phosphate buffer in methanol). LK-404 and LK-409 were prepared as described by Sollner et al [14, 16].

General procedure for N-(7-oxoacyl)-L-alanyl-D-isoglutamine benzyl esters ***5a–e***

To a stirred solution of 4 mmol 7-oxoalcanoic acid and 1.37 g (4 mmol) L-alanyl-D-isoglutamine benzyl ester hydrochloride [18] in 15 mL DMF 1.16 g (4.2 mmol) diphenylphosphoryl azide (DPPA) [27] and 1.11 mL (8 mmol) triethylamine were added at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 48–70 h. After the usual work-up procedure, recrystallization from methanol/ether yielded the pure compounds.

General procedure for N-(7-oxoacyl)-L-alanyl-D-isoglutamines ***6a–e***

N-(7-Oxoacyl)-L-alanyl-D-isoglutamines were prepared in 86–96% yield from 2 mmol *N*-(7-oxoacyl)-L-alanyl-D-isoglutamine benzyl esters by treatment with 120 mg 10% palladium/carbon and hydrogen in 70 mL methanol for 20–60 min. The catalyst was removed by filtration and the filtrate evaporated. The solid obtained was recrystallized from methanol/ether or acetone.

Pharmacology. Immunorestoration test

For the immunorestoration test, ICR (Institute of Cancer Research) derived female mice, aged 6 weeks, 23–27 g in weight, were used. The test substance or vehicle (distilled water) was administered ip to groups of 10 mice on days 1, 3 and 5, with immunosuppressant cyclophosphamide (25 mg/kg, po) administered on days 2, 4 and 6. One hour after the last immunosuppressant dose, the mice were challenged with a suspension of *C albicans* sufficient to result in up to 90% mortality within 10 days in the vehicle (distilled water) and cyclophosphamide-treated control groups. In these experiments, the within-group variability differs for only one animal in both the blank control treated with distilled water (vehicle) (survival of eight or nine mice) and in the vehicle and cyclophosphamide-treated control group (survival two or three mice); consequently, the number of potential survival is six animals in all tested groups (table II). The procedure for test substance evaluation involved the administration of four dose levels (0.1, 1, 10, 100 mg/kg) plus concurrent vehicle control. The survival of three animals more than in the group of cyclophosphamide is considered significant, and possibly a result of immunorestoration activity.

Table II. The influence of LK-409 derivatives on immunorestoration in mice.

Compound	*Dose (mg/kg)*	*Survival (No actual/No potential[b])*
LK-409	0.1	2/6
	1	0/6
	10.0	1/6
	100.0	2/6
LK-450	0.1	0/6
	1	0/6
	10.0	0/6
	100.0	1/6
LK-451	0.1	0/6
	1	0/6
	10.0	0/6
	100.0	0/6
LK-452	0.1	0/6
	1	1/6
	10.0	2/6
	100.0	2/6
LK-404	0.1	0/6
	1	3/6
	10.0	3/6
	100.0	4/6
LK-453	0.1	1/6
	1	1/6
	10.0	2/6
	100.0	3/6
LK-405	0.1	2/6
	1	3/6
	10.0	3/6
	100.0	3/6
Azimexone	100.0	3/6

[a]Survival number for group treated with test substance and cyclophosphamide-survival number for cyclophosphamide-treated group; [b]survival number for blank control-survival number for cyclophosphamide-treated group.

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