Journal of Enzyme Inhibition and Medicinal Chemistry, April 2005; 20(2): 115–121

Anticandidal properties of N-acylpeptides containing an inhibitor of glucosamine-6-phosphate synthase

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(Received 11 June 2004; in final form 25 July 2004)

Abstract

A series of N-acyl peptides **1**–**9**, containing an inhibitor of glucosamine-6-phosphate synthase have been synthesised and tested against *Candida* strains. N-Acylated peptides inhibit glucosamine-6-phosphate synthase in cell free extracts from *Candida albicans*. Antifungal activities of the tested compounds correlated with their lipophilic properties. Peptides acylated with decanoic acid were found to be the most potent in the series. N-decanoylpeptides also showed activity against *Candida albicans* Gu5 resistant mutant with Cdr1 and Cdr2 drug extrusion proteins that causes MDR by an active efflux mechanism.

Keywords: *Antifungal activity, glucosamine-6-phosphate synthase inhibition, N-acyl peptides*

Introduction

Fungal opportunistic infections caused mainly by the pathogenic fungus *Candida albicans* have increased in recent years particularly in immunocompromised and debilitated patients [1]. Currently used drugs for treatment of invasive fungal infections are limited to Amphotericin B and its novel formulations, including also a group of azoles and flucytosin [2]. None of these drugs, however, meets all the criteria required for good antifungal agents. Therefore a strong need exists for novel antifungals to treat these life threatening infections. In our research programme directed on novel antifungal agents we focused our attention on glutamine analogues, i.e. inhibitors and inactivators of fungal glucosamine-6-phosphate synthase (GlcN-6-P synthase; EC 2.6.1.16) [3]. This enzyme plays an important role in the biosynthesis of a number of amino sugar-containing macromolecules, including chitin and mannoproteins in fungi, peptidoglycan and lipopolisaccharides in bacteria [4]. Accordingly, GlcN-6-P synthase has been proposed as a potential target for antibacterial and especially antifungal agents [5]. A number of glutamine analogues acting as inhibitors and inactivators of GlcN-6-P synthase of fungal origin have been developed in our laboratory. It was shown that, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) is the most potent, selective and effective inactivator of fungal GlcN-6-P synthase [6]. A number of peptides composed of FMDP and protein and non-protein amino acids showed potent antifungal activity [7]. These peptides are taken up into yeast cells by active transport mediated by permeases, specific for di- and tripeptides [8]. However, fungi can easily develop a phenotypic resistance to these peptides, since peptide permeases are not essential for fungal growth [9]. The resistance is associated with failure of the peptides to penetrate the fungal cells. Obviously, such resistance phenomenon results in a decrease in effectiveness of antifungal peptides. In order to overcome this problem we have undertaken the chemical modification of FMDP-peptides aimed at the preparation of more lipophilic FMDP-peptides that can enter the fungal cells by free diffusion. An approach directed at changing the lipophilicity of active compounds is often used to improve the bioavailability and

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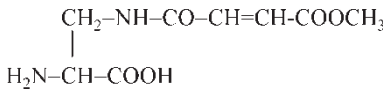
ISSN 1475-6366 print/ISSN 1475-6374 online © 2005 Taylor & Francis Ltd
DOI: 10.1080/14756360400009317

the cell membrane passage of molecules [10]. In the present communication we report the synthesis of a series of N-acylated peptides including also several N-acyl FMDP derivatives.

1. Ac–FMDP **4**. Ac–Nva–FMDP **7**. Ac–Lys(Ac) – Nva–FMDP
2. Hex–FMDP **5**. Hex–Nva–FMDP **8**. Hex–Lys(Hex) – Nva–FMDP
3. Dec–FMDP **6**. Dec–Nva–FMDP **9**. Dec–Lys(Dec) – Nva–FMDP

where FMDP



Moreover, we also present here the evaluation of these compounds as GlcN-6-P synthase inhibitors, the testing of their *in vitro* antifungal activity, determination of their lipophilicity and toxicity.

Experimental

Chemistry

N^2-Acetyl, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***1***. N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride [11] (0.5 g, 2 mmol) was dissolved in methanol (5 ml) cooled to 0°C and NEt_3 (0.6 ml, 4 mmol) was added. Then the reaction mixture was treated with acetic anhydride (1.32 ml, 2.2 mmol). The reaction mixture was stirred at the same temperature for 4 h, evaporated to dryness, dissolved in cold acetone, and filtered. The organic layer was treated with ethyl ether to precipitate the white solid which was filtered off to give the title compound **1** (0.42 g, 83%). M.p. 150–152° C. NMR(DMSO-d_6) $\delta = 2.27$ (3H, s), 3.7 (3H, s), 4.1 (2H,m), 4.3 (1H, m), 6.6 (2H, ABq, J = 16 Hz), 6.8 (1H, m), 7.27(1H, m); $[\alpha]_D + 4.2°$ (c = 1, MeOH). Anal. Calc. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5,46; N, 10.85. Found: C, 46.32; H, 5.20; N, 10.61%.

N^2-hexanoyl, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***2***. N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride (0.5 g, 2 mmol) and NEt_3 (0.6 ml, 4 mmol) were dissolved in a water-THF solution (1:1, 20 ml) at 0°C. Then N-succinimidoyl hexanoate (0.426 g, 2 mmol) was added with stirring and the reaction mixture was left overnight. The solvents were removed *in vacuo*. The residue was dissolved in 10 ml of water, acidified with 1 M $KHSO_4$ to pH = 2 and extracted with ethyl acetate (3 × 30 ml). The organic phase was dried and the solvent was concentrated leaving an oily residue which was crystallized from ethyl acetate-ethyl ether to give **2** as a white solid (0.47 g, 76% yield). M.p. 100–103°C. NMR($CDCl_3$) $\delta = 0.9$ (3H, t, J = 7 Hz), 1.2–1.4 (4H, m), 1.5–1.7 (2H, m), 2.2 (2H, m), 3.7 (3H, s), 3.6–3.8 (2H, m), 4.3(1H, m), 6.1 (1H, brs), 6.6 (2H, ABq, J = 16 Hz), 7.1 (1H, br.s); $[\alpha]_D + 5.7°$ (c = 1, MeOH). Anal. Calc. for $C_{14}H_{22}N_2O_6$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.85; H, 6.87; N, 8.69%.

N^2-decanoyl, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***3***. Starting from N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride (0.5 g, 2 mmol), and NEt_3 (0.6 ml, 4 mmol) dissolved in methanol (10 ml), and N-succinimidoyl decanoate (0.54 g, 2 mmol) dissolved in THF (5 ml), compound **3** was prepared as described for the preparation of **2**. Yield 0.5 g, 68%. M.p. 96–97°C. NMR($CDCl_3$) $\delta = 0.9$ (3H,t), 1.3–1.8 (14H, m), 2.15 (2H, m), 3.5–3.7 (2H, m), 3.8 (3H, s), 4.4 (1H, m), 6.3 (1H, br.s), 6.86 (2H, ABq, J = 16 Hz), 7.2 (1H, br.s). $[\alpha]_D + 6.2°$, (c = 1, MeOH) Anal. Calc. for $C_{18}H_{30}N_2O_6$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.32; H, 7.90; N, 7.65%.

N^2-(N-acetyl-L-norvalyl),N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***4***. N^2-L-Norvalyl,N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride [12] (0.35 g, 1 mmol) was dissolved in cold methanol (10 ml) and NEt_3 (0.3 ml, 2 mmol) was added. Then acetic anhydride (0.2 ml, 2 mmol) was added with stirring and the reaction mixture was kept overnight. The solvent was removed *in vacuo*, the residue was dissolved in water (20 ml), acidified with 1 M $KHSO_4$, and extracted with ethyl acetate (3 × 20 ml). The organic extract was dried over $MgSO_4$ and evaporated to give **4** as a solid. Yield 0.22 g, 62%. M.p. 124–126°C. NMR($CDCl_3$) $\delta =$ 0.95 (3H, t), 1.3 (2H, m), 1.8 (2H, m), 1.95 (3H, s), 3.75 (3H, s), 3.6-3.9 (2H,m), 4.4 (1H, m), 5.1 (1H, m), 5.8 (1H, brs), 6.8 (2H, ABq, J = 16.0 Hz), 7.1 (1H, brs). $[\alpha]_D - 12.5°$, (c = 1, MeOH). Anal. Calc. for $C_{15}H_{23}N_3O_7$: C, 50.42; H, 6.44; N, 11.76. Found: C, 50.68; H, 6.48; N, 11.45%.

N^2-(N-hexanoyl-L-norvalyl),N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***5***. This was prepared from

the peptide, N^2-L-norvalyl, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride and N-succinimidoyl hexanoate as described for compound **2**. Yield 0.27 g, 66%. M.p. 170–172°C. NMR($CDCl_3$) δ = 0.95 (6H, m), 1.2–1.8 (10H,m), 2.1(2H, m), 3.65 (3H, s), 3.6–3.85 (2H, m), 4.3(1H, m), 5.6 (1H, m), 5.8 (1H, m), 6.8(2H, ABq, J = 16 Hz), 7.2 (1H, brs). $[\alpha]_D$ − 19.6°, (c = 1, MeOH). Anal. Calc. for $C_{19}H_{31}N_3O_7$: C, 55.20; H, 7.50; N, 10.16. Found: C, 55.52; H, 7.12; N, 9.88%.

N^2-(N-decanoyl-L-norvalyl),N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***6***. This compound was prepared from the dipeptide N^2-L-norvalyl, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride and N-succinimidoyl decanoate analogously as described for compound **3**. Yield 0.286 g, 61%. M.p. 140–143°C. NMR($CDCl_3$) δ = 0.9 (6H, m), 1.3–2.0 (18H, m), 2.1 (2H, m), 3.6 (3H, s), 3.7–3.9 (2H, m), 4.3 (1H, m), 5.7 (1H, m), 5.9 (1H, m), 6.8 (2H, ABq, J = 16 Hz), 7.1 (1H, brs). $[\alpha]_D$ − 15.5°, (c = 1, MeOH). Anal. Calc. for $C_{23}H_{39}N_3O_7$: C, 58.85; H, 8.31; N, 8.95. Found: C, 58.59; H, 8.12; N, 8.62%.

N^2-(N^α,N^ω-diacetyl-L-lysyl-L-norvalyl), N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***7***. This compound was prepared from N^2-(L-lysyl-L-norvalyl), N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid dihydrochloride [13] (0.51 g, 1 mmol), NEt_3 (0.45 ml, 3 mmol) and acetic anhydride (0.22 ml, 2 mmol) using the procedure applied to the preparation of **4**. Yield 0.35 g, 68%. M.p. 132–133°C. NMR($CDCl_3$) d = 0.95 (3H, m), 1.3–1.8(8H, m), 2.0 (3H,s), 2.1(3H, s), 3.6–3.8 (2H, m), 3.65 (3H, s), 3.9–4.0 (4H, m), 4.1 (1H, m), 4.3 (1H, m), 5.6 (1H, m), 5.8 (1H, m), 6.8 (2H, ABq, J = 16 Hz), 7.2 (1H, brs), 7.4(1H, brs). $[\alpha]_D$ − 14.5°, (c = 1, MeOH). Anal. Calc. for $C_{23}H_{37}N_5O_9$: C, 52.37; H, 7.02; N, 13.28. Found: C, 52.67; H, 6.78; N, 13.31%.

N^2-(N^α,N^ω-dihexanoyl-L-lysyl-L-norvalyl), N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***8***. This compound was prepared from N^2-(L-lysyl-L-norvalyl), N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid dihydrochloride (0.51 g, 1 mmol), NEt_3 (0.45 ml, 3 mmol) and N-succinimidoyl decanoate (0.42 g, 2 mmol) according to the procedure used for the preparation of **2**. Yield 0.4 g, 62%. M.p. 184–188°C. NMR(DMSO-d_6) δ = 0.9–1.0 (9H, m), 1.1–1.4 (16H, m), 1.5–1.7 (6H, m), 1.8–2.0 (2H, m),2.1–2.4 (4H, m), 3.1–3.4 (2H, m), 3.6(3H, s), 3.7–3.8 (2H, m), 4.2 (1H, m), 5.7(1H, m),6.1 (1H, m), 6.8 (2H, ABq, J = 16 Hz),6.8 (1H, m), 7.2 (1H, brs), 8.1 (1H, brs). $[\alpha]_D$ − 14.6°, (c = 1, MeOH). Anal. Calc. for $C_{31}H_{53}N_5O_9$: C, 58.21; H, 8.29; N, 10.95. Found: C, 57.92; H, 8.22; N, 10.62%.

N^2-(N^α,N^ω-Didecanoyl-L-lysyl-L-norvalyl), N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid ***9***. This compound was prepared from N^2-(L-lysyl-L-norvalyl), N^3-(4-methoxyfumaroyl)-L-2,3-diamino propanoic acid dihydrochloride (0.51 g, 1 mmol), NEt_3 (0.45 ml, 3 mmol) and N-succinimidoyl hexanoate (0.54 g, 2 mmol) analogously as described for the preparation of compound **2**. Yield 0.51 g, 66%. M.p.166–168°C. NMR(DMSO-d_6) δ = 0.8–1.0 (9H, m),), 1.1–1.5 (22H, m), 1.5–1.7 (14H, m), 1.8–2.0 (4H, m),2.1–2.4 (4H, m), 3.1–3.4 (2H, m), 3.6(3H, s), 3.7–3.8 (2H, m), 4.2 (1H, m), 5.7(1H, m),6.1 (1H, m), 6.8 (2H, ABq, J = 16 Hz),6.8 (1H, m), 7.2 (1H, brs), 8.1 (1H, brs). $[\alpha]_D$ − 16.2°, (c = 1, MeOH). Anal. Calc. for $C_{39}H_{69}N_5O_9$: C, 62.45; H, 9.18; N, 9.32. Found: C, 62.66; H, 8.91; N, 9.04%.

Preparation of the crude enzyme (cell free extract) and determination of glucosamine-6-phosphate synthase activity

Candida albicans glucosamine-6-phosphate synthase cell free extract was prepared by the previously described procedure [14]. The concentration of glucosamine-6-phosphate was determined by the modified Elson-Morgan procedure [15]. Glc-6-P synthase activity was assayed as described previously,[14] using 15 mM D-fructose-6-phosphate, 10 mM L-glutamine, 50 mM potassium phosphate buffer (pH 7.0), inhibitor at an appropriate concentration and 0.1–0.2 μM Glc-6-P synthase (final protein concentration 0.1 mg/ml) in a total volume of 2 ml incubation mixture.

Inhibition of glucosamine-6-phosphate synthase

Standard incubation mixtures containing 40 μl of a cell free extract of Glc-6-P synthase (0.1 mg/ml of protein), 240 μl of 25 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 40 μl of 7.5 mM D-fructose-6-phosphate, 40 μl of 10 mM L-Gln and 40 μl of inhibitor at various concentrations in a total volume of 0.4 ml were incubated at 37°C for 30 min. Then the IC_{50} assays were performed as described previously, in two independent experimental versions. The first one was done immediately after addition of all components, the second one containing only cell free extract and inhibitor solution was preincubated for 30 min and the remaining components containing D-fructose-6-phosphate, L-glutamine and buffer solutions were added to the incubation mixture. All the measurements were done in triplicate.

In Vitro *activity*

Minimal inhibitory concentrations (MIC) were determined by a microbroth serial dilution technique (96-well microtitre plates) in YNB modified medium containing 1.7 g of YNB without amino acids and ammonium sulfate (Difco), 0.4 g of sodium glutamate, 10 mg of L-histidine, 20 mg of L-methionine, 20 mg of L-tryptophan and 10 mg of D-glucose in 1000 ml of distilled water. The medium was inoculated with various *Candida* strain cells from overnight cultures on the same medium to a concentration of 10^5 cfu/ml and incubated at 37°C for 24 h. MIC's were defined as the lowest concentration of the compound that prevented visible growth of yeasts. Results were determined using a turbidimetric method at 660 nm.

Membrane affinity measurements

An HPLC column with a stationary phase, 1-myristoyl-2-[13-carbonylimidazolide-tridecanoyl]sn-3-glycerophospholine(lecithin-imidazolide) bonded to silicapropylamine with the unreacted propylamine moieties end-capped with C10 and C3 alkyl chains (IAM.PC.DD2) was purchased from Regis Technologies (Morton Grove, IL, USA). The column was 3 cm × 4.6 mm; particle diameter 12 μm; pore diameter 300 Å. The chromatographic system consisted of a Model L-6200 A pump, Model L-4250 UV/VIS detector and a Model D-2500 integrator (all from Merck – Hitachi, Vienna, Austria). 0.1 M Sorensen buffer (K_2HPO_4/KH_2PO_4, pH 7.2)/acetonitrile eluent in proportion 97.5:2.5, 95:5, 90:10, 85:15, 80:20, 70:30, 65:35, 60:40, 50:50 was used as a mobile phase. The injection volume was 10 μl of an acetonitrile solution. The flow rate was 1 ml/min and the samples were detected at 220 nm. The capacity factors k'_{IAM} were calculated assuming that the dead volume of the column was the signal given by 50 μg/ml citric acid solution. A standard, commercially available statistical package for regression analysis was employed on a personal computer.

In Vitro *cytotoxic evaluation*

Conditions used for human promyelocytic leukemia cell line HL-60 growth were the same as described elsewhere [16]. Cells of the required density were seeded and different concentrations of the tested compounds were added. The experiments were carried out in a controlled (5% CO_2), humidified atmosphere at 37°C. The cytotoxic activity (IC_{50}) values were defined as the concentration of the inhibitor causing 50% inhibition of cell growth as measured after 72 h by cell counting with a Coulter Counter Z2 (Coulter Electronics, Ltd., UK).

Results and discussion

Synthesis

The syntheses (Scheme 1) started with N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride (FMDP), the dipeptide N^2-L-norvalyl, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid hydrochloride and the tripeptide N^2-(L-lysyl-L-norvalyl), N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid dihydrochloride which were allowed to react with acetic anhydride under standard conditions. The corresponding N-acetyl derivatives **1**, **4** and **7** were obtained in good yields after crystallization. On the other hand, the desired N-hexanoyl and N-decanoyl derivatives of FMDD, dipeptide and tripeptide were acylated using the corresponding active esters. Thus, hexanoic and decanoic acids were activated with dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (HOSu) [17] to give the active ester with good yield (>90%). Acylation reactions were performed in water-THF or methanol-THF solutions to furnish the desired compounds **2**, **5**, **8**, **3**, **6** and **9** respectively in moderate yields. Compounds containing the decanoyl residue formed gelatinous solutions during extraction with ethyl acetate and were sparingly soluble in water.

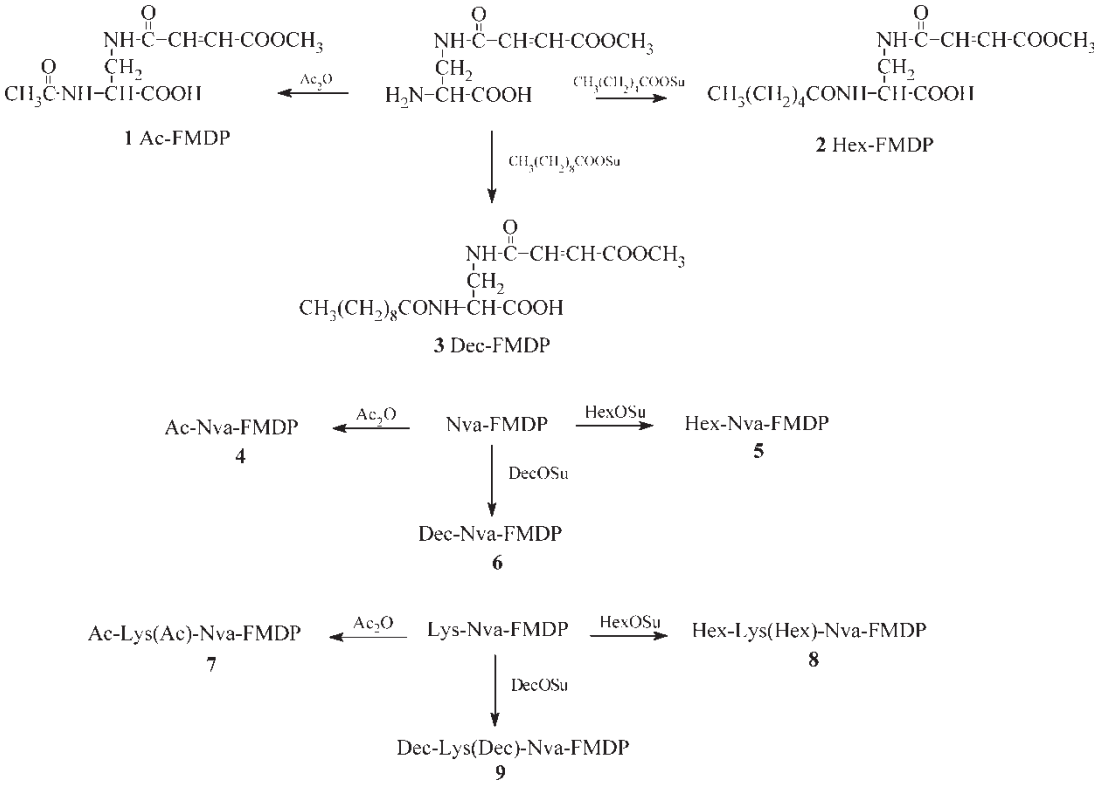
All new compounds were fully characterized by ^1H NMR and elemental analyses.

GlcN-6-P synthase inhibition studies

Compounds **1**, **4**, **7**, **2**, **5** and NvaFMDP (used for comparative purposes) were tested as inhibitors of a cell free extract of *Candida albicans* GlcN-6-P synthase. Data presented (Table I) confirm that a 30 min preincubation of a cell free extract of GlcN-6-P synthase with N-acyl compounds increased the inhibitory activity (IC_{50}) of all the tested compounds towards GlcN-6-P synthase. On the other hand, IC_{50} values measured for the same compounds when tested immediately after addition of all required components were much higher when compared to those obtained in the preincubation experiments. It should be noted that a preincubation time favours hydrolysis of peptide bonds by intracellular peptidases thus liberating FMDP, a GlcN-6-P synthase inhibitor. In the case of NvaFMDP practically the same IC_{50} values were observed when experiment was run with or without preincubation. It is suggested that FMDP was quickly released from the peptide upon intracellular hydrolysis mediated by peptidases.

Anticandidal activity and membrane affinity

The N-acylcompounds **1**–**9** were tested against a variety of *Candida* species; *Candida albicans* Gu4 (fluconazole sensitive), *Candida albicans* Gu5 (a gift from professor J. Morschhauser, University



Scheme 1. Synthesis of novel N-acylcompounds **1**–**9**.

of Wurzburg, Germany) and a number of *Candida* clinical isolates. The data presented in Table II clearly demonstrates that anticandidal activity strongly depends on the length of the acyl residues. It is generally accepted that only peptides with positively charged a N-terminal α-amino group and a free C-terminal carboxyl group are transported by bacterial peptide permeases into cells [18]. However, it not absolutely true for fungal permeases. The presence of multiple peptide permeases in the eukaryotic organism *Candida albicans* has been demonstrated and evidence was found for at least two peptide transport system in this species, the first one specific for di- and tripeptides and the second one specific for oligopeptides [8]. Therefore, not only are free peptides taken up by fungal permeases but also N-acetyl ones. Transport of N-acetylated or even N-Boc methionine-containing peptides has been reported into a methionine-lysine auxotroph of *Candida albicans* [19]. On the other hand the transport of N-acetyl peptides with alanine or glycine residues is completely prevented into *Candida albicans* 6406 strain [20]. These discrepancies may suggest the existence of specific, structural requirements for transport of N-acylated peptides into fungal cells.

Table I. Inhibition of GlcN-6-P synthase activity by N-acyl compounds.

	IC_{50} (mM)	
Compound	Preincubation	Without preincubation
AcFMDP **1**	0.15	1.8
AcNvaFMDP **4**	0.05	1.7
AcLys(Ac)NvaFMDP **7**	0.1	2.0
HexFMDP **2**	0.1	2.0
HexNvaFMDP **5**	0.08	1.52
NvaFMDP	0.05	0.07

We have found that the N-acetylcompounds **1**, **4** and **7** (i.e. AcFMDP, AcNvaFMDP and AcLys(Ac)NvaFMDP) exerted only poor antifungal activity. Therefore, they are probably not transported by fungal permeases and can not enter the cells through diffusion. Compounds acylated with hexanoic acid, **2**,**5** and **8**, showed better anticandidal properties. The most active compounds contain the decanoic acid residue as can be seen in **3**, **6** and **9** However, due to the poor solubility of **9** in the medium, low reproducibility of the MIC's for *C. albicans* Gu4 was obtained. Of the tested compounds only **9** showed noticeable activity against all strains tested. Hence, it was obvious that the length of the acyl chain was the factor influencing the anticandidal activity of FMDP compounds. The same correlation was observed when N-acyl compounds were tested against a series of clinical isolates of *Candida* species (Table III). The length of the acyl

Table II. Activity of compounds **1**–**9** against selected *Candida* strains and their membrane affinity.

Compound	MIC(μg/ml) *C. albicans* Gu4	MIC(μg/ml) *C. albicans* Gu5 (cdr1/cdr2)	log k'_{IAM}
AcFMDP **1**	3000	3000	ND*
AcNvaFMDP **4**	250	1000	ND
AcLys(Ac)NvaFMDP **7**	500	>2000	0.09
HexFMDP **2**	1000	1000	0.06
HexNvaFMDP **5**	31.25	1000	0.68
HexLys(Hex)NvaFMDP **8**	250	1000	1.40
DecFMDP **3**	62.5	250	1.71
DecNvaFMDP **6**	15.6	125	2.59
DecLys(Dec)NvaFMDP **9**	<3.9–31.25 >	62.5	3.43

*ND = not determined.

residue apparently has a favourable effect on the anticandidal activity of the tested compounds. These compounds in general, were less active against a variety of clinical isolates than those of laboratory species as presented in Table II. The clinical species, according to general opinion, are less sensitive to the action of antimicrobial drugs than their laboratory counterparts.

It is noticeable that *C. parapsilosis*, one of the clinical isolates tested, was slightly more sensitive to the action of N-acyl compounds than other species. Interestingly, compound **9**, containing a decanoic acid residue has also showed acceptable activity against a multidrug resistant yeast strain of *Candida albicans* Gu5, containing the drug effluxing proteins Cdr1p and Cdr2p. The observed anticandidal activity suggests the involvement of a diffusion phenomenon on uptake of N-acyl compound into the cells.

The affinity of a drug for biological membranes is an important factor facilitating its diffusion into the cells. The ability of the N-acyl derivatives to diffuse into the cells may be determined using an HPLC chromatographic column IAM (immobilized artificial membrane) constituting a chromatographic stationary phase that mimics the fluid cell membrane. The retention time determined for the examined compounds and expressed as $\log k'_{IAM}$ values (Table II) may be approximately used as a measure of their lipophilic properties [21,22]. In fact, the $\log k'_{IAM}$ value, comprises not only the lipophilicity characteristic but also other interactions with membrane-like hydrogen bond formation, electrostatic interaction etc. Therefore, the $\log k'_{IAM}$ values reflects rather membrane affinity than lipophilic properties. Comparison of the anticandidal activity of the N-acylated compounds with their membrane affinity expressed as $\log k'_{IAM}$ values has indicated the correlation between activity and membrane affinity. It is apparent from inspection of Table II that as the length of the acyl substituent increases, the $\log k'_{IAM}$ and antifungal activity also increase, suggesting the influence of lipophilic properties of the examined compounds on their diffusion into the cells. Therefore, compound **9**, presumably the most lipophilic compound exerted also the highest anticandidal activity.

Cytotoxic evaluation

The results obtained for the selected N-acyl compounds clearly show the lack of toxic effect towards human tumour cells (Table IV). The high concentration of compounds make them safe and nontoxic for the tested human cells, therefore, they may also be regarded as nontoxic for normal human cells.

Table III. Anticandidal activity of N-acylcompounds **1**–**9**.

Compound	MIC (μg/ml) *C. famata*	*C. glabrata*	*C. humicola*	*C. crusei*	*C. parapsilosis*	*C. tropcalisi*	*C. albicans ATCC10261*
AcFMDP **1**	1500	1000	500	1000	500	1500	2000
AcNvaFMDP **4**	250	750	500	250	125	500	250
AcLys(Ac)NvaFMDP **7**	250	500	250	250	125	500	250
HexFMDP **2**	500	500	250	500	250	1000	1000
HexNvaFMDP **5**	250	250	125	250	125	1000	500
HexLys(Hex)NvaFMDP **8**	125	125	62.5	125	62.5	500	250
DecFMDP **3**	125	500	500	125	62.5	250	62.5
DecNvaFMDP **6**	125	250	250	125	62.5	250	125
DecLys(Dec)NvaFMDP **9**	62.5	125	125	62.5	31.5	125	62.5

Table IV. *In vitro* cytotoxic activity of examined compounds against HL-60 human promyelocytic leukemia cells.

Compounds	HL-60 IC_{50} (mM) ± SEM
AcNvaFMDP **4**	10% inhibition at 500 mM
AcLys(Ac)NvaFMDP **7**	20% inhibition at 500 mM
HexNvaFMDP **5**	25% inhibition at 500 mM
HexLys(Hex)NvaFMDP **8**	~600
DecFMDP **3**	~230
DecNvaFMDP **6**	250 ± 65
DecLys(Dec)NvaFMDP **9**	415 ± 70

Conclusions

We have synthesised new, N-acylated compounds having GlcN-6-P synthase inhibitors in their structures. The compounds containing a decanoic acid residue show the highest antifungal properties out of all the compounds tested. Their antifungal activity may be correlated with lipophilic properties. It is suggested that these compounds may enter the cells via free diffusion. These compounds also show negligible toxicity against a human leukemia cells. Further work is underway aimed at the design of novel, more water soluble compounds with GlcN-6-P synhase inhibitors which are able to penetrate the fungal cells via free diffusion.

Acknowledgements

This research was supported by the State Committee for Scientific Research (KBN), Grant No. 6 P05F 005 21. The authors are indebted to Dr. Maria Bontemps-Gracz for cytotoxic studies.

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