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Amide and Ester Derivatives of N^3-(4-Methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic Acid: The Selective Inhibitor of Glucosamine-6-phosphate Synthase

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Abstract—Several amide and ester derivatives of a glutamine analogue, N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid (FMDP) (**1**–**8**), were synthesized and evaluated for the inhibitory activity in regard to glucosamine-6-phosphate synthase from *Candida albicans*. The syntheses were accomplished by the reaction of N^2-*tert*-butoxycarbonyl-N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid (BocFMDP) with the corresponding amines to give the FMDP amides (**1**–**4**) or with alkyl halides to give corresponding esters of FMDP (**5**–**8**). Among the synthesized compounds, the acetoxymethyl ester of FMDP was the most active inhibitor of the enzyme. Its IC_{50} value compared to that of FMDP (4 μM) was equal to 11.5 μM. The methyl and allyl esters and the *N*-hexyl-*N*-methyl-amide of FMDP exhibited a moderate enzyme inhibitory activity.

Introduction

Selective inhibitors of glucosamine-6-phosphate synthase, EC 2.6.1.16, the novel generation glutamine analogues, are a new group of potential antimicrobial agents.[1–5] The selectivity of inhibitory action of these compounds allowed us to consider glucosamine-6-phosphate synthase (GlcN-6-P synthase) as a new target for antifungal compounds (for review, see ref 6). The enzyme is a key one in the biosynthesis of glucosamine-containing microbial cell-wall macromolecules: peptidoglycan in bacteria and mannoproteins and chitin in fungi. Specific inactivation of this enzyme by rationally designed glutamine analogues causes the inhibition of cell-wall biosynthesis, resulting in bactericidal and fungicidal effect.[4,7,8] Representative for this group of compounds is N^3-(4-methoxyfumaroyl) - (*S*) - 2,3 - diaminopropanoic acid (FMDP),[3,5] a potent and specific inhibitor of GlcN-6-P synthase from *Candida albicans,* which acts in an irreversible manner as an active site directed agent.[9–11] However, FMDP itself, as an amino acid analogue, is poorly transported into fungal cells by rather specific amino acid permeases and thus exhibits only moderate antifungal activity.[12] This disadvantage was overcome by an application of the portage (carrier) transport concept.[13–15] Following this strategy, FMDP-peptides were synthesized. These compounds due to the broad substrate spectrum of peptide permeases were effectively taken up by the cells and subsequently hydrolyzed by cytoplazmatic peptidases, with generation of a free FMDP inside the cells. FMDP-oligopeptides exhibited excellent antifungal activity[16–20] and were non-toxic to mammalian cells.[21] However, the ease with which microbial cells can 'switch off' peptide permeases is an obvious reason for the development of resistance. In order to overcome this problem, we have undertaken the studies on chemical modifications of FMDP molecule, aimed at the construction of lipophilic derivatives that might be able to penetrate into the cells by free diffusion. In this paper the structure–enzyme inhibitory activity relationship of FMDP derivatives was studied.

It should be noted that FMDP is a relatively small molecule and affords little possibility of structural modifications not affecting the enzyme inhibitory properties of this compound.[3–5] Especially, the reactive part of the fumaroyl moiety, directly interacting with the catalytic Cys-1 residue at the enzyme active site (conjugate addition),[7,9] should not be modified.

Abbreviations: Boc, *tert*-butoxycarbonyl; DBU, 1,8-diazabicycklo[5,4,0] undec-7-ene; DCC, *N*,*N*-dicyclohexylo-carbodiimide; DPPA, diphenyl azidophosphate; Et_3N, triethylamine; FMDP, N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid; GlcN-6-P, glucosamine-6-phosphate; SuNOH, *N*-hydroxysuccinimide.

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In turn, amino and carboxyl groups of the diaminopropanoic acid residue are important determinants of binding ability of a glutamine analogue to the enzyme.[4] Their modification should not impair this ability if stable derivatives are to be obtained. Electrostatic, hydrogen bonding and steric effects should thus be considered. In this respect, very limited data concerning the modification of amino and carboxyl groups of FMDP are available. It seems, however, that there is a very little chance for effective modification of an amino group. *N*-acetyl FMDP is a very poor inhibitor of GlcN-6-P synthase and *N*-acetyl-Gln cannot replace glutamine as an enzyme substrate in the amino group transfer reaction. On the other hand, modification of the carboxyl group gave more optimistic results. FMDP methyl ester[17] and dipeptide FMDP-Nva[21] retained partially the inhibitory activity of the parent compound. Thus we have decided to explore the possibility of modification of the carboxyl group for the construction of lipophilic FMDP analogues with GlcN-6-P synthase inhibitory activity. Some of these derivatives can be also of interest as diffusable lipophilic prodrugs, able to generate the free FMDP, upon the enzymatic cleavage inside fungal cells.

We have synthesized the series of lipophilic FMDP derivatives, modified at the carboxyl group. Amides (**1**–**4**) and esters (**5**–**8**) were examined in regard to their enzyme inhibitory potency. The structures of the compounds are shown in Figure 1. In the case of FMDP amides, we have intentionally chosen substituents with methyl group at the nitrogen atom, in order to obtain stable compounds, not susceptible to degradation by amidases in a biological environment. For comparison, we synthesized one amide, lacking a methyl group at the nitrogen atom.

Results and Discussion

Chemistry

The synthesis of compounds **1**–**8** was performed using standard synthetic procedures.[22–24] N^2-*tert*-Butoxycarbonyl-N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid[5,25] was coupled with *N*-methylisopropylamine, *N*-methylpropylamine, *N*-methylhexylamine and *sec*-butylamine, applying the active esters (*N*-hydroxysuccinimide esters) or the DPPA methods to give the FMDP amides **1**–**4** (Fig. 2). Application of the DPPA method allowed to considerably increase a final yield of these compounds. The FMDP esters **5** and **6** were obtained upon reaction of BocFMDP with allyl bromide or methyl iodide in the presence of diisopropylethylamine (Fig. 3). The FMDP derivatives **1**–**6** were prepared as trifluoroacetate salts.

In the case of of the FMDP acyloxymethyl esters synthesis (**7** and **8**), BocFMDP was treated with an excess of an appropriate halomethyl ester, in the presence of 1,8-diazabicycklo[5,4,0]undec-7-ene (DBU), to form the corresponding acyloxymethyl ester (Fig. 4). The reaction proceeds in a nonpolar solvent, such as benzene, to give final compounds **7** and **8** in good yields (Table 1). Esterification was carried out as follows. A mixture of BocFMDP, DBU, and halomethyl esters in benzene was refluxed for 2 h and the DBU-hydrohalides (DBUHX) were filtered off. The products **7** and **8** were purified on the Silicagel column with methylene chloride as a mobile phase and isolated as their crystalline trifluoroacetate salts. The halomethyl esters were prepared by treating the corresponding acid halide with paraformaldehyde.[26]

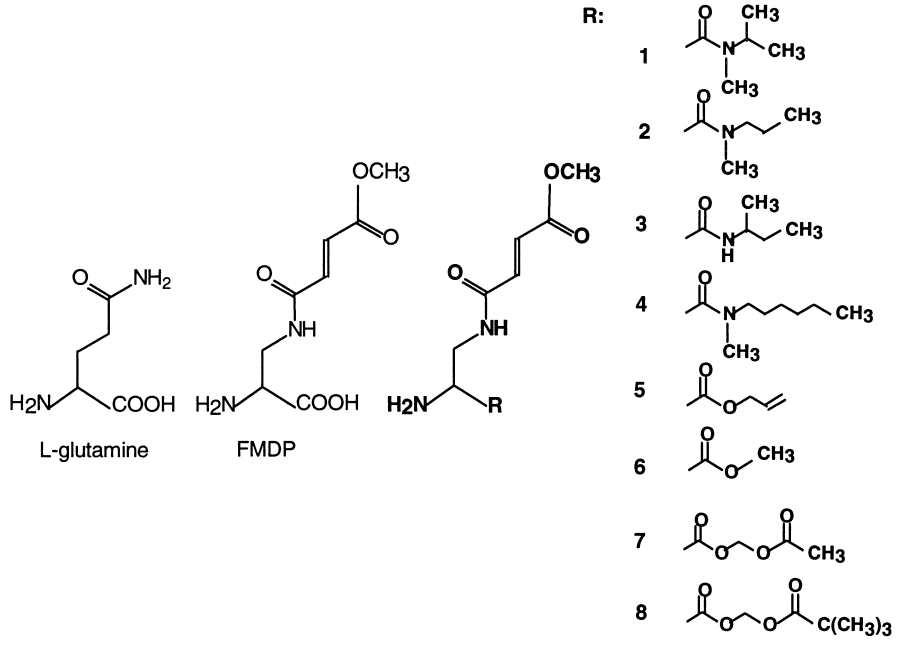


Figure 1. The structures of glutamine, FMDP and synthesized derivatives.

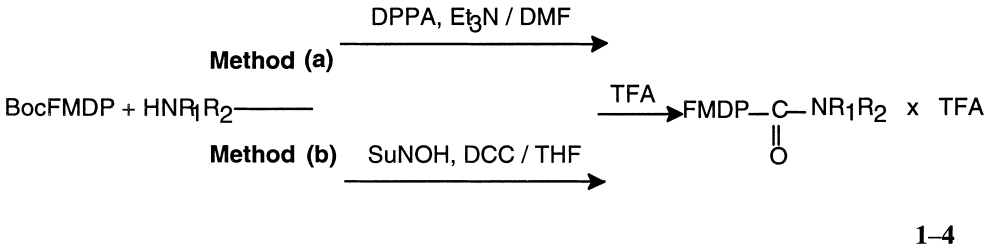


Figure 2. General route of FMDP amides synthesis (**1**–**4**) where R_1 and R_2 are: -$CH(CH_3)_2$ and -CH_3 for compound **1**, -$(CH_2)_2CH_3$ and -CH_3 for **2**, -$CH(CH_3)$–CH_2CH_3 and -H for **3**, -$(CH_2)_5CH_3$ and -CH_3 for **4**.

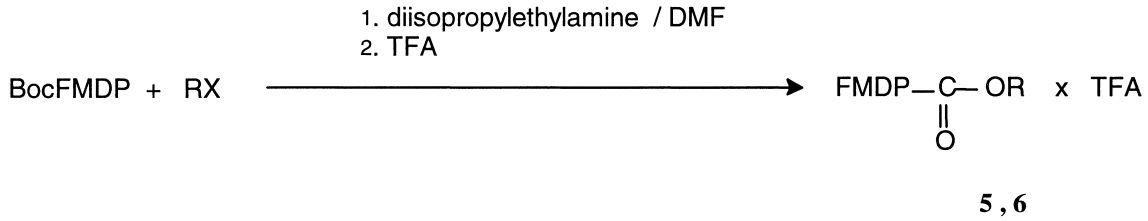


Figure 3. General route of FMDP esters synthesis (**5** and **6**), where R and X are: -CH_2–$CH{=}CH_2$ and -Br for compound **5**, -CH_3 and -I for **6**.

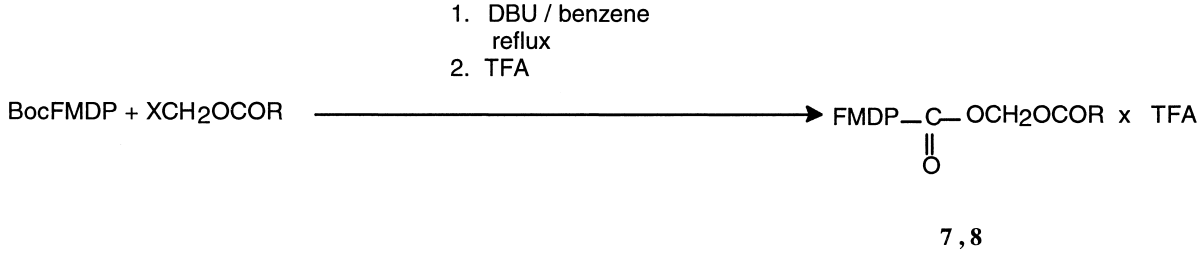


Figure 4. General route of FMDP acyloxymethyl esters synthesis (**7** and **8**), where R and X are: -CH_3 and -Br for compound **7**, -$C(CH_3)_3$ and -Cl for **8**.

Enzyme inhibitory activity

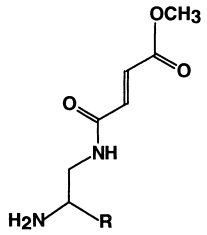
All of the obtained FMDP analogues were tested for inhibition of *C. albicans* glucosamine-6-phosphate synthase activity. The ability of compounds **1**–**8** to inhibit this enzyme was measured by determing a concentration of inhibitor causing 50% inhibition of the enzyme. The results are summarized in Table 2.

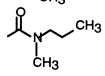
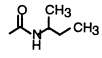
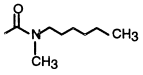
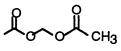
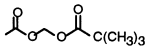
Acyloxymethyl esters of FMDP (**7** and **8**) were found to be the most active inhibitors of GlcN-6-P synthase. Their IC_{50} values are only a few-fold higher than that of FMDP (4 μM) and amounted to 11.5 and 15.6 μM for acetoxymethyl ester and pivaloyloxymethyl ester, respectively. These compounds, being potential prodrugs, might be hydrolyzed to FMDP in the buffer. The determination of their stability in the assay buffer was thus needed. Application of chromatographic methods (TLC) and NMR spectroscopy allowed us to confirm that only the acetoxymethyl ester of FMDP (**7**) is stable in the assay buffer (at least for 24 h, at 30 °C). The pivaloyloxy-methyl ester (**8**) is unstable in these conditions and is partially hydrolyzed to FMDP. Surprisingly enough, the methyl ester of FMDP (**6**) with IC_{50} value of 300 μM, shows a moderate inhibitory potency.

On the other hand, among the FMDP amide derivatives (**1**–**4**), the *N*-hexyl-*N*-methyl amide (**4**) was found to be the most active inhibitor, with IC_{50} value of 500 μM, comparable to that found for the FMDP allyl ester **5** (520 μM). *N-iso*-Propyl-*N*-methyl amide (**1**) was slightly less active. The significant decrease of inhibitory activity was observed in the case of compound **3** (IC_{50} = 2910 μM). It has been found that the inhibitory potency of compounds **1**–**7** follow the order: **7**≫**6** > **4**, **5** > **1** > **2** > **3**.

Compounds **1**–**7** were tested as inactivators of the enzyme and kinetic parameters of inactivation were compared to those found for FMDP. Incubation of *C. albicans* glucosamine-6-phosphate synthase with those compounds in the absence of glutamine led to irreversible inactivation of the enzyme. The time course of inactivation caused by compounds **4** and **6** are shown in Figures 5 and 6. When values for the apparent rate constants of inactivation (k_{app}), calculated from the slope of lines in Figures 5 and 6, were plotted against inactivator concentration, hyperbolic curves were obtained (not shown). Such a pattern is consistent with the formation of a reversible complex before inactivation (eq (1)):

Table 1. Analytical data of FMDP amides **1**–**4** and esters **5**–**8**[a]



No.	R	Yield (%)	$[\alpha]_{578}$ (c 1, MeOH)	Mp (°C)	Formula	Anal. calcd C; H; N Found C; H; N
1		69	−10.8	94–96	$C_{14}H_{22}N_3O_6F_3$	43.64; 5.71; 10.91 42.08; 5.97; 9.35
2		80	−2.0	(−)	$C_{14}H_{22}N_3O_6F_3$	43.64; 5.71; 10.91 (−)
3		73	−4.8	150–152	$C_{14}H_{22}N_3O_6F_3$	43.64; 5.71; 10.91 44.39; 6.14; 9.14
4		81	−2.0	68–71	$C_{17}H_{28}N_3O_6F_3$	47.78; 6.56; 9.84 45.47; 6.39; 10.05
5		57	−5.6	77–78	$C_{13}H_{18}N_2O_7F_3$	42.05; 4.85; 7.55 41.64; 5.38; 7.47
6		78	−16.7	53–55	$C_{11}H_{15}N_2O_7F_3$	38.37; 4.36; 8.14 38.13; 4.16; 8.89
7		77	+12.0	121–123	$C_{13}H_{17}N_2O_9F_3$	38.84; 4.27; 6.97 39.24; 4.47; 6.91
8		75	+4.0	96–98	$C_{16}H_{23}N_2O_9F_3$	43.28; 5.22; 6.31 46.29; 5.66; 5.92

[a]All compounds were prepared and analyzed as trifluoroacetate salts; FMDP amides (**1**–**4**) were made by the DPPA method; (−) means that TFA salts of FMDP derivatives form highly hygroscopic, amorphous powders without reproducible melting points and turning fast into oil, not suitable for elemental analysis.

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [E^*I] \overset{k_2}{\rightarrow} EI^* \quad (1)$$

where [E*I] is the enzyme–inhibitor complex and EI* is the irreversibly modified enzyme. Assuming that [I]≫[E] and that the reversible complex is at all times in equilibrium with enzyme and inhibitor, the equation derived by Meloche[27] can be applied (eq (2)):

$$\tau = 1/[I] \times (T \times K_{inact}) \; + \; T \quad (2)$$

Table 2. Inhibition of glucosamine-6-phosphate synthase from *Candida albicans* by FMDP and derivatives **1**–**8**

Compounds	IC_{50}[a] (μM)
FMDP	4 ± 0.5
1	970 ± 80
2	1400 ± 120
3	2910 ± 95
4	500 ± 35
5	520 ± 48
6	300 ± 22
7	11.5 ± 1.5
8	15.6 ± 2.1

[a]IC_{50}, the 50% inhibitory concentrations of compounds that inhibit the enzyme activity by 50% of the control value. Values are the means of at least three independent estimations ± sd.

or

$$\frac{1}{k_{app}} = \frac{K_{inact}}{k_2} \times \frac{1}{[I]} + \frac{1}{k_2} \quad (3)$$

where:

K_{inact} = [E][I]/[E*I]
T = the minimum inactivation half time at the infinite inhibitor concentration,
k_{app} = the apparent pseudo-first-order constant, $k_{app} = (\ln 2)/\tau$
τ = the inactivation half time at a given inhibitor concentration [I],
$k_2 = (\ln 2)/T$ = the inactivation constant at the infinite inhibitor concentration.

Kinetic parameters of inactivation calculated from eq (3) are summarized in Table 3. The lowest values of K_{inact} were observed for esters of FMDP: the acetoxymethyl (**7**) (596 μM), methyl (**6**) and allyl ester (**5**) (about 1400 μM for both), thus suggesting that these compounds exhibit the highest affinity for the enzyme active site,[28] although this is much lower than in the case of FMDP (5.13 μM). The reactivities of inhibitors reflected by k_2 values do not differ markedly and range from 0.34 min^{-1} for **1** to 0.83 min^{-1} for compound **2**. On the other hand, their inactivation ability expressed by

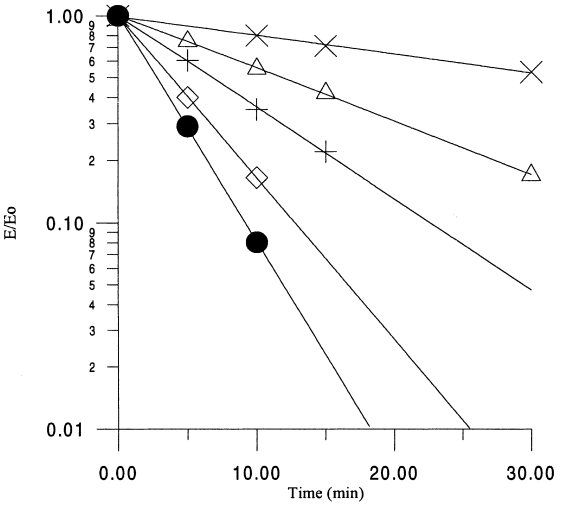


Figure 5. Inactivation of glucosamine-6-phosphate synthase from *Candida albicans* by methyl ester of FMDP (**6**) at concentrations of 1.5 mM (●), 0.75 mM (◇), 0.375 mM (+), 0.1875 mM (△), 0.09375 mM (×).

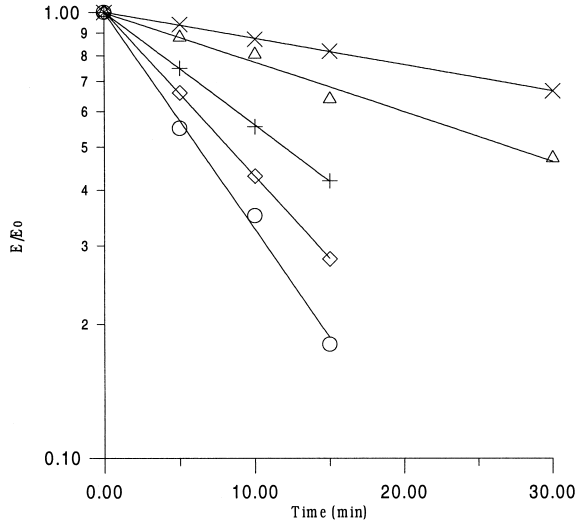


Figure 6. Inactivation of glucosamine-6-phosphate synthase from *Candida albicans* by *N*-hexyl-*N*-methylamide of FMDP (**4**) at concentrations of 2 mM (○), 1 mM (◇), 0.5 mM (+), 0.25 mM (△), 0.125 mM (×).

the ratio of k_2/K_{inact}[27] are in much broader range from 0.32 (M^{-1} s^{-1}) for **3** to 22.0 (M^{-1} s^{-1}) for compound **7** and follow the order: **7**≫**5** > **6** > **4** > **2** > **1** > **3**. Narrow range of k_2 values for FMDP derivatives **1**–**7** suggests that the reactivity of inhibitors do not differentiate their enzyme inhibitory potency. The primary role is played by the affinity of compounds to the enzyme. As shown in Figure 7, the correlation has been found between affinity of FMDP derivatives to glucosamine-6-phosphate synthase reflected by K_{inact} and their enzyme inhibition ability (a linear fit; coef of determination, R-squared = 0.95). When L-glutamine, 15 mM, was present in the incubation mixtures instead of D-fructose-6-phosphate, GlcN-6-P synthase was not inactivated by the FMDP derivatives studied by us. A protective effect of one of the enzyme substrates points at the

Table 3. Kinetic parametrs of inactivation of glucosamine-6-phosphate synthase from *Candida albicans* by FMDP and compounds **1**–**7**[a]

Compounds	k_2 (min^{-1})	K_{inac} (μM)	k_2/K_{inac} (M^{-1} s^{-1})
FMDP	0.477	5.13	1556
1	0.34	14,300	0.40
2	0.83	23,000	0.60
3	0.41	21,600	0.32
4	0.51	7800	1.09
5	0.73	1400	8.80
6	0.48	1350	5.95
7	0.79	596	22.0

[a]Values of kinetic constants are the means of at least three independent estimations. Standard deviations were lower than 5%.

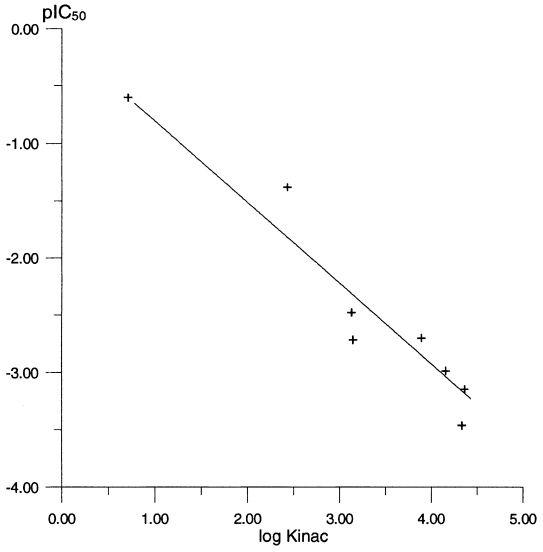


Figure 7. The relation between the affinity of FMDP and its derivatives (**1**–**7**) to glucosamine-6-phosphate synthase and their enzyme inhibitory activity. The full line represents a linear fit; coefficient of determination, R-squared = 0.95; $pIC_{50} = -\log pIC_{50}$.

competition between FMDP esters/amides and L-glutamine for the enzyme active center, as it was previously shown for FMDP.[10]

Conclusions

In summary: (1) Substitution at carboxyl group of FMDP provides lipophilic compounds with inhibitory activity towards glucosamine synthase. Ester and amide derivatives exhibit broad range of activities depending on their structures, with acetoxymethyl ester retaining to great extent the activity of modified FMDP. (2) The modification of FMDP at the carboxyl group has an impact on the affinity to the enzyme, which is an essential factor governing the enzyme inhibitory activity of the compounds. (3) The described derivatization of FMDP at carboxyl group apparently does not influence essentially the electrophilic properties of the compounds as expressed by their reactivity towards the enzyme. The reactivities of all compounds studied are comparable. Thus, the reactivity of electrophiles examined should not be an obstacle in their application as potential drugs,

because peptides with similarily reactive electrophile, FMDP, were evidenced to be effective in experimental candidiasis.[29] (4) Evidencing the enzyme inhibitory activity of carboxyl substituted FMDP derivatives suggests that these compounds might provide a solution to the drug uptake with omitting the otherwise indispensable portage transport.

Experimental

Chemistry

Melting points were measured in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded at 200 MHz on a Varian 360 instrument with Me_4Si as an internal reference. MS spectrum for compound **2** was recorded on a Quadrupolic Mass Spectrophotometer Trio-3 (FAB technique). Optical rotations were measured in a Polamat (Carl Zeiss Jena) polarimeter. TLC was carried out on Kieselgel 60 F 254 plates (Merck) in solvent system: AcOEt/MeOH/H_2O (5:1:0.75 v/v/v) for amides (**1**–**4**) and esters (**5** and **6**) of FMDP and ProOH/H_2O (7:3 v/v) for acyloxymethyl esters of FMDP (**7** and **8**). The location of spots was detected by spraying with ninhydrin, cerium sulphate reagents or by illumination with a UV lamp. N^2-*tert*-Butoxycarbonyl-N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid (BocFMDP) was synthesized according to the earlier described method[1,2] and was used for the preparation of compounds **1**–**8** (Figs 2–4). All amines (*N*-methylhexylamine, *N*-methylisopropylamine, *N*-methylpropylamine, and *sec*-butylamine, diisopropylethylamine, triethylamine) and halides (allyl bromide, methyl iodide) were purchased from Aldrich Chemical Co. All other chemicals were of the highest purity commercially available.

General procedure for synthesis of FMDP amides (1–4)

Method A. To a solution of BocFMDP (0.281 g, 0.889 mmol), triethylamine (0.26 mL, 1.867 mmol) and DPPA (0.21 mL, 0.978 mmol) in DMF (3 mL), a corresponding amine (0.978 mmol) was added (*N*-methylhexylamine, *N*-methylisopropylamine, *N*-methylpropylamine, *sec*-butylamine). The reaction mixture was stirred at room temperature overnight and diluted with ethyl acetate (7 mL). The organic layer was washed with saturated NaCl solution, $NaHCO_3$ (1 M), $KHSO_4$ (1 M) and water and dried over $MgSO_4$. After evaporation of the solvent, the residue was crystallized from diethyl ether–hexane and next dissolved in cold trifluoroacetic acid (4 mL) and kept at room temperature for 3 h. Excess TFA was evaporated in vacuo, the residue was triturated with diethyl ether and the precipitate was filtered off, dried in vacuo over KOH pellets. Compounds **1**, **3** and **4** were analyzed by ^{1}H NMR and elemental analysis. Hygroscopic, oily compound **2** was analyzed by ^{1}H NMR and mass spectroscopy.

***N*-Isopropyl-*N*-methylamide of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (1).** 0.236 g, (69% yield), mp 94–96 °C, $[\alpha]_{578}$ −10.8° (*c* 1, MeOH), R_f 0.51; ^{1}H NMR (DMSO) δ 0.99–1.25 (m, 6H, 2×CH_3), 2.75 (s, 1.5H, N–CH_3), 2.90 (s, 1.5H, N–CH_3), 3.40–3.71 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.15–4.3 (septet, 0.5H, N–CH), 4.42 (t, 1H, CH) 4.54–4.7 (septet, 0.5H, N–CH), 6.8 (dd, 2H, J = 15.4 Hz, CH=CH), 8.2 (br s, 3H, NH_3^+), 8.9 (t, 1H, NH). Found: C, 42.08; H, 5.97; N, 9.35; calcd for $C_{14}H_{22}N_3O_6F_3$: C, 43.64; H, 5.71; N, 10.91.

***N*-Methyl-*N*-propylamide of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (2).** 0.274 g, (80% yield), $[\alpha]_{578}$ −2.0° (*c* 1, MeOH), R_f 0.53; ^{1}H NMR (DMSO) δ 0.75–0.92 (dt, 3H, CH_3), 1.08–1.21 (dsekst, 2H, CH_2), 2.85 (s, 1.5H, N–CH_3), 3.09 (s, 1.5H, N–CH_3), 3.25–3.38 (m, 2H, N–CH_2), 3.50–3.70 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.41 (t, 1H, CH), 6.81 (dd, 2H, J = 15 Hz, CH=CH), 8.25 (br. s, 3H, NH_3^+), 8.9 (t, 1H, NH); MS *m*/*z* 272 ($M^+ + 1$), 255, 170, 101.

***N*-*sec*-Butylamide of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (3).** 0.250 g, (73% yield), mp 150–152 °C, $[\alpha]_{578}$ −4.8° (*c* 1, MeOH), R_f 0.53; ^{1}H NMR (DMSO) δ 0.76–0.80 (m, 3H, CH_3), 1.05 (t, 3H, CH_3), 1.36–1.49 (dqwint, 2H, CH_2), 3.43–3.61 (septet, 1H, N–CH), 3.65–3.72 (m, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.82 (t, 1H, CH), 6.80 (dd, 2H, J = 15.4 Hz, CH=CH), 8.27 (br s, 3H, NH_3^+), 8.38 (d, 1H, OC–NH), 8.79 (t, 1H, NH). Found: C, 44.39; H, 6.14; N, 9.14; calcd for $C_{14}H_{22}N_3O_6F_3$: C, 43.64; H, 5.71; N, 10.91.

***N*-Hexyl-*N*-methylamide of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (4).** 0.308 g, (81% yield), mp 68–71 °C, $[\alpha]_{578}$ −2.0° (*c* 1, MeOH), R_f 0.54; ^{1}H NMR ($CDCl_3$) δ 0.81–0.95 (dt, 3H, CH_3), 1.21–1.4 (m, 8H, 4×CH_2), 2.92 (s, 1.5H, N–CH_3), 3.21 (s, 1.5H, N–CH_3), 3.32–3.5 (m, 1H, N–CH_2), 3.6–3.78 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.92 (t, 1H, CH), 4.55–4.72 (m, 1H, N–CH_2), 6.8 (dd, 2H, J = 15.7 Hz, CH=CH), 7.02 (t, 1H, NH). Found: C, 45.47; H, 6.39; N, 10.05; calcd for $C_{17}H_{28}N_3O_6F_3$: C, 47.78; H, 6.56; N, 9.84.

Method B. To a solution of BocFMDP (0.20 g, 0.630 mmol) and *N*-hydroxysuccinimide (0.08 g, 0.690 mmol) in THF (4 mL), *N*,*N*′-dicyclohexylocarbodiimide (0.14 g, 0.690 mmol) in THF and a corresponding amine (0.690 mmol) were added. The reaction mixture was kept at room temperature overnight with stirring. After this time, the dicyclohexylourea was filtered off and the filtrate evaporated to a dryness. The residue was dissolved in ethyl acetate (7 mL) and washed with water, $KHSO_4$ (1 M), Na_2CO_3 (1 M) and saturated NaCl solution and dried over $MgSO_4$. The solvent was evaporated and a residue crystallized from mixture of diethyl ether–hexane. The removal of the Boc group from the obtained compound as above.

***N*-Methyl-*N*-propylamide of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (2).** 0113 g, (33% yield), $[\alpha]_{578}$ −2.2° (*c* 1, MeOH), R_f 0.53; ^{1}H NMR (DMSO) δ 0.73–0.90 (dt, 3H, CH_3), 1.09–1.22 (dsekstet, 2H, CH_2), 2.85 (s, 1.5H, N–CH_3), 3.07 (s, 1.5H, N–CH_3), 3.24–3.30 (m, 2H, N–CH_2), 3.52–3.71 (m., 2H, CH_2), 3.74 (s, 3H, OCH_3), 4.43 (t, 1H, CH),

6.80 (dd, 2H, $J=15$ Hz, CH=CH), 8.24 (br. s, 3H, NH_3), 8.88 (t, 1H, NH).

***N*-Hexyl-*N*-methylamide of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (4).** 0.148 g, (39% yield), mp 68–71 °C, $[\alpha]_{578}$ −2.0° (*c* 1, MeOH), R_f 0.54; ^{1}H NMR ($CDCl_3$) δ 0.81–0.95 (dt, 3H, CH_3), 1.26–1.38 (m, 8H, 4×CH_2), 2.93 (s, 1.5H, N–CH_3), 3.24 (s, 1.5H, N–CH_3), 3.32–3.5 (m, 1H, N–CH_2), 3.52–3.68 (m, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.92 (t, 1H, CH), 4.55–4.72 (m, 1H, N–CH_2), 6.89 (dd, 2H, $J=17.5$ Hz, CH=CH), 7.05 (t, 1H, NH).

General procedure for synthesis of FMDP esters (5 and 6)

To a solution of BocFMDP (0.200 g, 0.633 mmol), diisopropylethylamine (0.11 mL, 0.633 mmol) in DMF (3 mL), a corresponding halide (1.329 mmol) (allyl bromide, methyl iodide) was added. The reaction mixture was stirred at room temperature overnight and diluted with ethyl acetate (7 mL). The organic layer was washed with saturated NaCl solution, $NaHCO_3$ (1 M), $KHSO_4$ (1 M) and water and dried over $MgSO_4$. After evaporation of the solvent, the residue was diluted in a small amount of diethyl ether and purified on the Silicagel column with diethyl ether as a mobile phase. The organic layer was dried and concentrated under reduced pressure and next dissolved in cold trifluoroacetic acid (4 mL) and kept at room temperature for 3 h. Excess TFA was evaporated in vacuo, the residue was triturated with diethyl ether and the precipitate was filtered off, dried in vacuo over KOH pellets.

Allyl ester of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (5). 0.134 g, (57% yield), mp 77–78 °C, $[\alpha]_{578}$ −5.6° (*c* 1, MeOH), R_f 0.68; ^{1}H NMR (DMSO) δ 3.5–3.65 (m, 2H, CH_2), 3.74 (s, 3H, OCH_3), 4.23 (t, 1H, CH), 4.64–4.7 (m, 2H, CH_2=CH), 5.34–5.43 (m, 2H, CH_2), 5.87–6.00 (dqwintet, 1H, $CH_2=CH$), 6.80 (dd, 2H, $J=15.4$ Hz, CH=CH), 8.44 (br.s, 3H, NH_3^+), 8.88 (t, 1H, NH). Found: C, 41.64; H, 5.38; N, 7.47; calcd for $C_{13}H_{18}N_2O_7F_3$: C, 42.05; H, 4.85; N, 7.55.

Methyl ester of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (6). 0.170 g, (78% yield), mp 53–55 °C, $[\alpha]_{578}$ −15.7° (*c* 1, MeOH), R_f 0.83; ^{1}H NMR (DMSO) δ 3.41 (s, 3H, OCH_3), 3.5–3.65 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.22 (t, 1H, CH), 6.79 (dd, 2H, $J=15.6$ Hz, CH=CH), 8.45 (br.s, 3H, NH_3), 8.85 (t, 1H, NH). Found: C, 38.13; H, 4.16; N, 8.89; calcd for $C_{11}H_{15}N_2O_7F_3$: C, 38.37; H, 4.36; N, 8.14.

General procedure for synthesis of FMDP acyloxymethyl esters (7 and 8)

Halomethyl ester (pivaloyloxymethyl chloride, acetoxymethyl bromide) (1.067 mmol) in benzene (1 mL) was added to a solution of BocFMDP (0.28 g, 0.889 mmol) and DBU (0.133 mL, 0.889 mmol) in benzene (8 mL) and the mixture was refluxed with stirring for 2 h. After cooling, the mixture was diluted with diethyl ether (20 mL), the precipitate (DBUHX) was filtered and washed with diethyl ether or ethyl acetate. The filtrate and the washing were combined, washed with water, citric acid (0.5 M), $NaHCO_3$ (1 M), and water again, and dried over sodium sulphate. After evaporation of the solvent, the residue was diluted in a small amount of methylene chloride and purified on the Silicagel column with methylene chloride as a mobile phase. The organic layer was dried and concentrated under reduced pressure (bath temp 40 °C) and next dissolved in cold trifluoroacetic acid (4 mL) and kept at room temperature for 3 h. Excess TFA was evaporated in vacuo, the residue was triturated with diethyl ether and the precipitate was filtered off, dried in vacuo over KOH pellets.

Acetoxymethyl ester of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (7). 0.275 g, (77% yield), mp 121–123 °C, $[\alpha]_{578}$ +12.0° (*c* 1, MeOH), R_f 0.79; ^{1}H NMR (DMSO) δ 2.09 (s, 3H, CH_3), 3.53–3.70 (m, 2H, CH_2), 3.74 (s, 3H, OCH_3), 4.23 (t, 1H, CH), 5.76 (dd, 2H, $J=6.0$ Hz, CH_2), 6.80 (dd, 2H, $J=15.6$ Hz, CH=CH), 8.50 (br.s, 3H, NH_3), 8.85 (t, 1H, NH). Found: C, 39.24; H, 4.47; N, 6.91; calcd For $C_{13}H_{17}N_2O_9F_3$: C, 38.84; H, 4.27; N, 6.97.

Pivaloyloxymethyl ester of N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid trifluoroacetate (8). 0.296 g, (75% yield), mp 96–98 °C, $[\alpha]_{578}$ +4.0° (*c* 1, MeOH), R_f 0.82; ^{1}H NMR (DMSO) δ 1.16 (s, 9H, (CH_3), 3.5–3.65 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.26 (t, 1H, CH), 5.83 (dd, 2H, $J=6.0$ Hz, CH_2), 6.80 (dd, 2H, $J=15.6$ Hz, CH=CH), 8.50 (br.s, 3H, NH_3^+), 8.88 (t, 1H, NH). Found: C, 46.29; H, 5.66; N, 5.92; calcd for $C_{16}H_{23}N_2O_9F_3$: C, 43.28; H, 5.22; N, 6.31.

Enzyme purification

C. albicans GlcN-6-P synthase was purified to apparent homogeneity using the previously described procedure.[30] Protein was determined by the method of Bradtford.[31]

Determination of glucosamine-6-phosphate synthase activity

The standard incubation mixture contained: D-fructose-6-phosphate (15 mM), L-glutamine (10 mM), EDTA (1 mM), potassium phosphate buffer (25 mM, pH 6.5), inhibitor at an appropriate concentration and enzymatic protein (0.005–0.01 mg mL^{-1}) in a total volume of 0.4 mL. The mixtures were incubated at 37 °C for 30 min. The reaction was stopped by heating at 100 °C for 1 min. The concentration of glucosamine-6-phosphate was determined by the modified Elson–Morgan procedure.[32]

Inactivation of glucosamine-6-phosphate synthase

Incubation mixtures containing: glucosamine-6-phosphate synthase from *C. albicans* (0.005–0.01 mg), albumine (1 mg/mL), phosphate buffer (25 mM, pH 6.5), EDTA (1 mM), D-fructose-6-phosphate (15 mM) and inactivators at various concentrations in a total volume of 1 mL, were incubated at 25 °C. For protection experiments, D-fructose-6-phosphate was substituted by L-glutamine, 15 mM. To follow inactivation of the enzyme, aliquots

(200 μL) were withdrawn from the reaction mixture and applied at the top of small, 1-mL columns packed with Sephadex G-25 (equilibrated with the 25 mM potassium phosphate buffer pH 6.5) and centrifuged (500×*g* for 1 min at 4 °C). Under these conditions the unbound inhibitor was separated from the enzyme and the protein was recovered in clean test-tubes. Appropriate effluent aliquots were used for the determination of the residual enzyme activity using the standard assay method.

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