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RESEARCH ARTICLE

# The synthesis and biological activity of lipophilic derivatives of bicine conjugated with $N^3$ -(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP)—an inhibitor of glucosamine-6-phosphate synthase

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## Abstract

A series of bis- $N,N$ -(2-hydroxyethyl)glycine (bicine) derivatives, conjugated with an inhibitor of glucosamine-6-phosphate synthase, have been synthesized and their lipophilic and antifungal properties have been tested. The obtained compounds demonstrated higher lipophilicity than free inhibitor (FMDP) and, in consequence, an increased potential to cross the cytoplasmic membrane. All the tested compounds show better antifungal activity than parent compound.

**Keywords:** Antifungal agents, FMDP derivatives, prodrug

## Introduction

Infections caused mainly by human pathogenic fungi are regarded as one of the most important problems to be solved in modern chemotherapy. Only a very limited number of antifungal chemotherapeutics are in clinical use.<sup>1</sup> One of the possible solutions to overcome this problem is to consider an exploitation of new antifungal targets, for example enzymes involved in the biosynthesis pathway of the fungal cell wall.<sup>2</sup> Glucosamine-6-phosphate synthase (GlcN-6-P synthase, EC 2.6.1.16)<sup>3</sup> catalyses the formation of D-glucosamine-6-phosphate and therefore is one of the enzymes required for the biosynthesis of glucosamine-containing cell wall macromolecules: lipopolysaccharides and peptidoglycan in bacteria and mannoproteins and chitin in fungi.<sup>4</sup> Inhibition of this enzyme in fungal cells leads to morphological changes and lysis,<sup>5,6</sup> whereas in mammalian cells temporary depletion of enzyme activity is not lethal due to long half-life of GlcN-6-P synthase and rapid expression of the genes encoding it.<sup>7</sup> Thus, GlcN-6-P synthase has been proposed as a potential target for designing new antimicrobial agents.<sup>8</sup> There are many inhibitors of GlcN-6-P

synthase that are analogues of glutamine. Representative for this group of compounds is  $N^3$ -(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), one of the most potent and selective inhibitors of the enzyme.<sup>9,10</sup> FMDP is a non-peptide amino acid, therefore is poorly transported into fungal cells by rather specific amino acid permeases and exhibits only moderate antifungal activity. High polarity of the inhibitor effectively hampered its transport inside the cells. In order to overcome this problem, we have modified FMDP molecule, aimed at the construction latent and lipophilic derivatives that might be able to penetrate into the cells by free diffusion. Following uptake, the modifying group could be removed intracellularly. Modification of the carboxyl group involves the formation of latent esters, such as acetoxymethyl ester.<sup>11</sup> That approach is very common in penicillin group.<sup>12</sup> On the other hand, formation of prodrugs by modification of the amino group is relatively rare and difficult.<sup>13</sup> In our approach, we have tried to apply bicine, that is, bis- $N,N$ -(2-hydroxyethyl)glycine as an acylating agent of FMDP molecule. Bicine amide underwent, at physiological pH, a facilitated cyclization (with  $t_{1/2}=3$ h) to a

4-(2-hydroxyethyl)morpholin-2-one with the releasing of ammonia molecule. Formation of the six-membered ring is the driving force in this reaction. The  $t_{1/2}$  for hydrolysis of glycinamide at physiological pH was determined to be ~7 years.<sup>14</sup> Therefore, the amide bond between bicine and FMDP should be cleaved as a result of intramolecular alcoholysis, thus leading to the formation of FMDP and 4-(2-hydroxyethyl)morpholin-2-one. To increase stability and lipophilicity of prodrug, esterification of hydroxyl groups in bicine was carried out, according to the published procedure.<sup>15</sup> The fully modified FMDP prodrug should penetrate into the fungal cells by free diffusion, and inside, the ester bonds should be cleaved by intracellular enzymes followed by cyclization of the bicine residue to a 4-(2-hydroxyethyl)morpholin-2-one and generation of free FMDP (Scheme 3). As a consequence of that reaction, inhibition of fungal cells should be observed. Moreover, we have also evaluated lipophilicity of the novel prodrugs by measuring their affinity to biological membranes.

## Experimental

### Chemistry

All reagents were purchased from Aldrich Chemical Co. Thin layer chromatography (TLC) was performed using Merck aluminum backed plates (Kieselgel 60 F<sub>254</sub>) and visualized by ultraviolet (UV) light. Separations by column chromatography were achieved using silica gel (0.063–0.200 mm). MS spectrum was recorded on a Quadrupole Mass Spectrophotometer Trio-3 (FAB technique). <sup>1</sup>H NMR spectra were recorded on a Varian Unity Plus spectrometer operating at 500 MHz using deuterated solvent (CDCl<sub>3</sub>). Chemical shifts are given in part per million (ppm) relative to internal standard tetramethylsilane.

#### *Tert-butyl ester of bis-N,N-(2-hydroxyethyl)glycine (1)*

A solution of tert-butyl bromoacetate (5.40 g, 27.70 mmol) and diethanolamine (11.60 g, 110.80 mmol) in anhydrous methylene chloride (DCM, 250 mL) was stirred at room temperature for 18 h. The reaction mixture was washed with water (4 × 50 mL), and the organic layer dried over anhydrous sodium sulphate, followed by filtration and removal of the solvent *in vacuo* to yield **1** (4.05 g, 67%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.45 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 2.89 (t, 4H, *J* = 4.4 Hz, 2x(CH<sub>2</sub>N)). MS (FAB) *m/z*: 220 (MH<sup>+</sup>).

#### *General method for the preparation of bis-N,N-(2-acyloxyethyl)glycine trifluoroacetate (3a–d)*

A solution of compound **1** (0.20 g, 0.91 mmol), 4,4'-(dimethylamino)pyridine (DMAP, 0.11 g, 0.91 mmol) and appropriate carboxylic acid (0.91 mmol) in anhydrous DCM (15 mL) was cooled to 0°C. Dicyclohexylcarbodiimide (DCC, 0.206 g, 1.00 mmol) was added and the reaction mixture was stirred at 0°C for 30 min and then at room temperature for 12 h. The reaction mixture was filtered, the filtrate was washed with 0.5

N NaHCO<sub>3</sub> (3 × 10 mL), water (10 mL) and dried (MgSO<sub>4</sub>). The solvent was evaporated *in vacuo*. Tert-butyl ester of bis-*N,N*-(2-acyloxyethyl)glycine (**2a–d**) was further purified by silica gel column chromatography. Compounds **2a–d** were treated with TFA for 4 h at room temperature. Excess TFA was evaporated *in vacuo*, the residue was triturated with diethyl ether and the precipitate was filtered off and dried *in vacuo* over KOH pellets.

*Bis-N,N-(2-acetoxyethyl)glycine trifluoroacetate (3a)* Tert-butyl ester of bis-*N,N*-(2-acetoxyethyl)glycine (**2a**) was purified by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:7) to give **2a** (0.25 g, 91%). Compound **3a** was obtained as waxy solid (0.29 g, 97%). <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 2.05 (s, 6H, 2x(CH<sub>3</sub>C(O))); 3.01 (t, 4H, *J* = 5.8 Hz, 2x(CH<sub>2</sub>N)); 3.41 (s, 2H, NH<sub>2</sub>C(O)); 4.15 (t, 4H, *J* = 5.8 Hz, 2x(OCH<sub>2</sub>)). MS (FAB) *m/z*: 248 (MH<sup>+</sup>).

*Bis-N,N-(2-propionyloxyethyl)glycine trifluoroacetate (3b)* Tert-butyl ester of bis-*N,N*-(2-propionyl)glycine (**2b**) was purified by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:8) to give **2b** (0.23 g, 79%). Compound **3b** was obtained as waxy solid (0.265 g, 95%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.13 (t, 6H, *J* = 7.5 Hz, 2x(CH<sub>3</sub>)); 2.33 (q, 6H, *J* = 7.5 Hz, 2x(CH<sub>2</sub>)); 3.00 (m, 4H, 2x(CH<sub>2</sub>N)); 3.42 (s, 2H, NH<sub>2</sub>C(O)); 4.17 (m, 4H, 2x(OCH<sub>2</sub>)). MS (FAB) *m/z*: 276 (MH<sup>+</sup>).

*Bis-N,N-(2-butyryloxyethyl)glycine trifluoroacetate (3c)* Tert-butyl ester of bis-*N,N*-(2-butyryloxyethyl)glycine (**2c**) was purified by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:10) to give **2c** (0.23 g, 73%). Compound **3c** was obtained as waxy solid (0.269 g, 97%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.94 (t, 6H, *J* = 7.4 Hz, 2x(CH<sub>3</sub>)); 1.66 (q, 4H, *J* = 7.4 Hz, 2x(CH<sub>2</sub>)); 2.28 (t, 4H, *J* = 7.4 Hz, 2x(CH<sub>2</sub>C(O))); 2.98 (t, 4H, *J* = 6 Hz, 2x(CH<sub>2</sub>N)); 3.39 (s, 2H, NH<sub>2</sub>C(O)); 4.13 (t, 4H, *J* = 6 Hz, 2x(C(O)CH<sub>2</sub>)). MS (FAB) *m/z*: 304 (MH<sup>+</sup>).

*Bis-N,N-(2-benzoyloxyethyl)glycine trifluoroacetate (3d)* Tert-butyl ester of bis-*N,N*-(2-benzoyloxyethyl)glycine (**2d**) was purified by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:12) to give **2d** (0.225 g, 58%). Compound **3d** was obtained as waxy solid (0.23 g, 92%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 3.25 (t, 4H, *J* = 5.8 Hz, 2x(CH<sub>2</sub>N)); 3.58 (s, 2H, NH<sub>2</sub>C(O)); 4.46 (t, 4H, *J* = 5.8 Hz, 2x(C(O)CH<sub>2</sub>)); 7.36–7.58 (m, 6H,); 7.99–8.05 (m, 4H). MS (FAB) *m/z*: 372 (MH<sup>+</sup>).

#### *General method for the preparation of FMDP acyloxyalkyl esters (6a–f)*

To a solution of BocFMDP (**4**) (2.00 g, 6.33 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.96 g, 6.33 mmol) and sodium iodide (0.35 g, 1.90 mmol) in anhydrous acetonitrile (25 mL), the corresponding 1-chloroalkyl ester (**5a–f**) was added (7.60 mmol) and the mixture was refluxed with stirring for 5 h. The solvent was evaporated, the residue was diluted with ethyl acetate, washed

with 0.5 N NaHCO<sub>3</sub> (2 × 20 mL), water (20 mL) and dried (MgSO<sub>4</sub>). The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (ethyl acetate:petroleum ether, 1:2). The product was treated with TFA for 2 h at room temperature. 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<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid trifluoroacetate (6a)* Yield 1.42 g (56%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 2.15 (s, 3H, (O)CCH<sub>3</sub>); 3.82 (s, 5H, OCH<sub>3</sub>, NCH<sub>2</sub>); 4.45 (m, 1H, CH); 5.81 (dd, 2H, *J* = 5 Hz, OCH<sub>2</sub>O); 6.90 (ABq, 2H, *J* = 15 Hz, CH=CH). MS (FAB) *m/z*: 389 (MH<sup>+</sup>).

*(Isobutyryloxy)methyl ester of N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid trifluoroacetate (6b)* Yield 1.58 g (58%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.18 (d, 6H, *J* = 6.5 Hz, 2x(CH<sub>3</sub>)); 2.64 (m, 1H, CH); 3.80 (s, 5H, OCH<sub>3</sub>, NCH<sub>2</sub>); 5.75 (dd, 2H, *J* = 5 Hz, OCH<sub>2</sub>O); 6.86 (ABq, 2H, *J* = 15 Hz, CH=CH). MS (FAB) *m/z*: 417 (MH<sup>+</sup>).

*(2-Phenylacetoxy)methyl ester of N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid trifluoroacetate (6c)* Yield 1.91 g (63%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 3.71 (s, 2H, CH<sub>2</sub>); 3.80 (s, 5H, OCH<sub>3</sub>, NCH<sub>2</sub>); 4.42 (m, 1H, CH); 5.75 (dd, 2H, *J* = 5 Hz, OCH<sub>2</sub>O); 6.86 (ABq, 2H, *J* = 15 Hz, CH=CH); 7.32 (m, 5H, Ar). MS (FAB) *m/z*: 465 (MH<sup>+</sup>).

*[(2-Ethylbutanoyl)oxy]methyl ester of N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid trifluoroacetate (6d)* Yield 2.17 g (75%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.86 (t, 6H, *J* = 7.4 Hz, 2x(CH<sub>3</sub>)); 1.62 (m, 4H, 2x(CH<sub>2</sub>)); 2.35 (m, 1H, CH); 3.79 (s, 5H, OCH<sub>3</sub>, NCH<sub>2</sub>); 4.42 (m, 1H, CH); 5.78 (dd, 2H, *J* = 5.6 Hz, OCH<sub>2</sub>O); 6.88 (ABq, 2H, *J* = 15 Hz, CH=CH). MS (FAB) *m/z*: 445 (MH<sup>+</sup>).

*1-Acetoxypropyl ester of N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid trifluoroacetate (6e)* Yield 1.44 g (53%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.99 (m, 3H, CH<sub>3</sub>); 1.84 (m, 2H, CH<sub>2</sub>); 2.11 (s, 3H, CH<sub>3</sub>C(O)); 3.80 (s, 5H, OCH<sub>3</sub>, NCH<sub>2</sub>); 4.45 (m, 1H, CH); 5.65 (m, 1H, OCHO); 6.86 (m, 2H, CH=CH). MS (FAB) *m/z*: 417 (MH<sup>+</sup>).

*1-[(Ethoxycarbonyl)oxy]ethyl ester of N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid trifluoroacetate (6f)* Yield 1.44 g (51%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.35 (m, 3H, CH<sub>3</sub>); 1.58 (m, 2H, CH<sub>2</sub>); 3.80 (s, 5H, OCH<sub>3</sub>, NCH<sub>2</sub>); 4.28 (m, 2H, CH<sub>2</sub>); 4.45 (m, 1H, CH); 5.78 (m, 1H, OCHO); 6.86 (m, 2H, CH=CH). MS (FAB) *m/z*: 433 (MH<sup>+</sup>).

**General method for the preparation of acyloxyalkyl ester of N<sup>2</sup>-(bis-N,N-(2-acyloxyethyl)glyciny)-N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8a-i)**

A solution of bis-N,N-(2-acyloxyethyl)glycine trifluoroacetate (**3a-d**) (1 mmol), triethylamine (0.20 g, 2 mmol) and

N-hydroxysuccinimide (0.114 g, 1 mmol) in tetrahydrofuran (THF) (10 mL) was cooled to 0°C and DCC (0.216 g, 1.05 mmol) was added. After 12 h, the dicyclohexylurea was filtered off and the filtrate was added drop wise to solution of corresponding acyloxyalkyl ester of FMDP (1 mmol) and triethylamine (0.20 g, 2 mmol) in THF (7 mL). The mixture was stirred at 0°C for 30 min, and then at room temperature for 14 h. The solvent was evaporated, the residue was dissolved in ethyl acetate (20 mL), washed with 0.5 N NaHCO<sub>3</sub> (2 × 15 mL), water (10 mL) and dried (MgSO<sub>4</sub>). The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (ethyl acetate:petroleum ether, 3:1). The product was dissolved in ethyl acetate and equimolar amount of 2.00 M HCl in diethyl ether was added. The precipitate was filtered off and dried *in vacuo* over KOH pellets.

*Acetoxymethyl ester of N<sup>2</sup>-(bis-N,N-(2-acetoxyethyl)glyciny)-N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8a)* Yield 0.23 g (41%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 2.09 (s, 6H, 2x(CH<sub>3</sub>C(O))); 2.12 (s, 3H, CH<sub>3</sub>C(O)); 2.88 (m, 4H, 2x(CH<sub>2</sub>N)); 3.31 (d, 2H, *J* = 5.4 Hz, NCH<sub>2</sub>C(O)); 3.78 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.10–4.35 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.70 (m, 1H, NHCH); 5.75 (dd, 2H, *J* = 5.6 Hz, OCH<sub>2</sub>O); 6.90 (ABq, 2H, *J* = 15.4 Hz, CH=CH). MS (FAB) *m/z*: 518 (MH<sup>+</sup>).

*Acetoxymethyl ester of N<sup>2</sup>-(bis-N,N-(2-propionyloxyethyl)glyciny)-N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8b)* Yield 0.28 g (48%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.13 (t, 6H, *J* = 7.5 Hz, 2x(CH<sub>3</sub>)); 2.12 (s, 3H, CH<sub>3</sub>C(O)); 2.36 (q, 4H, *J* = 7.5 Hz, 2x(CH<sub>2</sub>C(O))); 2.88 (m, 4H, 2x(CH<sub>2</sub>N)); 3.29 (s, 2H, NCH<sub>2</sub>C(O)); 3.78 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.15–4.35 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.70 (m, 1H, NHCH); 5.75 (dd, 2H, *J* = 5.6 Hz, OCH<sub>2</sub>O); 6.90 (ABq, 2H, *J* = 15.4 Hz, CH=CH). MS (FAB) *m/z*: 546 (MH<sup>+</sup>).

*Acetoxymethyl ester of N<sup>2</sup>-(bis-N,N-(2-butyryloethyl)glyciny)-N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8c)* Yield 0.28 g (46%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.94 (t, 6H, *J* = 7.3 Hz, 2x(CH<sub>3</sub>)); 1.64 (m, 4H, 2x(CH<sub>2</sub>)); 2.12 (s, 3H, CH<sub>3</sub>C(O)); 2.32 (t, 4H, *J* = 7.5 Hz, 2x(CH<sub>2</sub>C(O))); 2.89 (t, 4H, *J* = 5.6 Hz, 2x(CH<sub>2</sub>N)); 3.31 (s, 2H, NCH<sub>2</sub>C(O)); 3.78 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.14–4.30 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.72 (m, 1H, NHCH); 5.76 (dd, 2H, *J* = 5.6 Hz, OCH<sub>2</sub>O); 6.88 (ABq, 2H, *J* = 15.4 Hz, CH=CH). MS (FAB) *m/z*: 574 (MH<sup>+</sup>).

*Acetoxymethyl ester of N<sup>2</sup>-(bis-N,N-(2-benzoyloxyethyl)glyciny)-N<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8d)* Yield 0.28 g (41%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 2.09 (s, 3H, CH<sub>3</sub>C(O)); 3.18 (t, *J* = 5.4 Hz, 4H, 2x(CH<sub>2</sub>N)); 3.53 (s, 2H, NCH<sub>2</sub>C(O)); 3.72 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.53 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.58 (m, 1H, NHCH); 5.83 (dd, 2H, *J* = 5.6 Hz, OCH<sub>2</sub>O); 6.82 (ABq, 2H, *J* = 15.4 Hz, CH=CH); 7.35–7.90 (m, 10H). MS (FAB) *m/z*: 642 (MH<sup>+</sup>).



(*Isobutyryloxy*)methyl ester of *N*<sup>2</sup>-(*bis-N,N*-(2-acetoxyethyl)glycyl)-*N*<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8e) Yield 0.23 g (40%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.16 (d, 6H, *J* = 6.5 Hz, 2x(CH<sub>3</sub>)); 2.09 (s, 6H, 2x(CH<sub>3</sub>C(O))), 2.69 (m, 1H, CH); 2.98 (m, 4H, 2x(CH<sub>2</sub>N)); 3.75 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.10–4.40 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.69 (m, 1H, NHCH); 5.78 (dd, 2H, *J* = 5.4 Hz, OCH<sub>2</sub>O); 6.89 (ABq, 2H, *J* = 15.4 Hz, CH=CH). MS (FAB) *m/z*: 546 (MH<sup>+</sup>).

(2-Phenylacetoxy)methyl ester of *N*<sup>2</sup>-(*bis-N,N*-(2-acetoxyethyl)glycyl)-*N*<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8f) Yield 0.23 g (37%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 2.09 (s, 6H, 2x(CH<sub>3</sub>C(O))); 3.05 (m, 4H, 2x(CH<sub>2</sub>N)), 3.71 (s, 2H, CH<sub>2</sub>); 3.78 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.10–4.40 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.65 (m, 1H, NHCH), 5.75 (dd, 2H, *J* = 5.4 Hz, OCH<sub>2</sub>O); 6.88 (ABq, 2H, *J* = 15.4 Hz, CH=CH). MS (FAB) *m/z*: 594 (MH<sup>+</sup>).

[(2-Ethylbutanoyl)oxy]methyl ester of *N*<sup>2</sup>-(*bis-N,N*-(2-acetoxyethyl)glycyl)-*N*<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8g) Yield 0.26 g (43%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.87 (t, 6H, *J* = 7.4 Hz, 2x(CH<sub>3</sub>)); 1.57 (m, 6H, 2x(CH<sub>2</sub>)); 2.09 (s, 6H, 2x(CH<sub>3</sub>C(O))); 2.27 (m, 1H, CHC(O)); 2.89 (m, 4H, 2x(CH<sub>2</sub>N)); 3.30 (m, 2H, NCH<sub>2</sub>C(O)); 3.78 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.10–4.40 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.70 (m, 1H, NHCH); 5.80 (dd, 2H, *J* = 5.5 Hz, OCH<sub>2</sub>O); 6.90 (ABq, 2H, *J* = 15.6 Hz, CH=CH). MS (FAB) *m/z*: 574 (MH<sup>+</sup>).

1-Acetoxypropyl ester of *N*<sup>2</sup>-(*bis-N,N*-(2-acetoxyethyl)glycyl)-*N*<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8h) Yield 0.24 g (41%). <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.97 (m, 3H, CH<sub>3</sub>); 1.82 (m, 2H, CH<sub>2</sub>); 2.11 (s, 3H, CH<sub>3</sub>C(O)); 2.13 (s, 6H, 2x(CH<sub>3</sub>C(O))); 2.92 (m, 4H, 2x(CH<sub>2</sub>N)); 3.40 (m, 2H, NCH<sub>2</sub>C(O)); 3.79 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.10–4.35 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.69 (m, 1H, NHCH); 6.70 (m, 2H, OCHO); 6.80 (m, 2H, CH=CH). MS (FAB) *m/z*: 546 (MH<sup>+</sup>).

1-[(Ethoxycarbonyl)oxy]ethyl ester of *N*<sup>2</sup>-(*bis-N,N*-(2-acetoxyethyl)glycyl)-*N*<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (8i) Yield 0.23 g (38%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.32 (m, 3H, CH<sub>3</sub>); 1.58 (m, 2H, CH<sub>2</sub>); 2.09 (s, 6H, 2x(CH<sub>3</sub>C(O))); 2.89 (m, 4H, 2x(CH<sub>2</sub>N)); 3.28 (m, 2H, NCH<sub>2</sub>C(O)); 3.75 (s, 5H, OCH<sub>3</sub>, HNCH<sub>2</sub>); 4.10–4.35 (m, 4H, 2x((CH<sub>2</sub>OC(O))); 4.22 (m, 2H, CH<sub>2</sub>); 4.71 (m, 1H, NHCH); 6.75 (m, 1H, OCHO); 6.90 (m, 2H, CH=CH). MS (FAB) *m/z*: 562 (MH<sup>+</sup>).

#### Determination of the affinity to the artificial biological membrane

Interactions between the tested compounds and immobilized artificial membrane were investigated using HPLC column IAM.PC.DD2 (Regis Technologies, Inc., Morton Grove, IL).<sup>16</sup> The column dimensions were 3 cm × 4.6 mm, particle diameter 10 μm and pore

width 300 Å. The chromatographic system consisted of a UV/vis detector Model G1315B, Vacuum degasser Model G1322A, Quaternary pump Model G1311A (Agilent Technologies 1200 Series, Palo Alto, CA). 0.1 M Sørensen buffer (K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>, pH 7.2)/acetonitrile was used in proportions 50:50, 60:40, 65:35, 70:30, 75:25, 80:20 (v/v) as mobile phase. The injection volume was 10 μL; the flow rate was 1 mL/min; the samples were detected at 254 nm. The dead volume of the column was determined by the retention time of citric acid (aqueous solution, 50 μg/mL) and was used to calculate capacity factors *k'*<sub>IAM</sub>. The dependence of log*k'*<sub>IAM</sub> versus concentration of acetonitrile was linear. The log*k'*<sub>IAM,0</sub> was obtained by extrapolation of logarithmic capacity factor to concentration of acetonitrile equal to 0. A standard, commercially available statistical package for regression analysis was applied on a personal computer.

#### Antifungal susceptibility tests

Minimal inhibitory concentrations (MICs) of the examined compounds were determined by the serial 2-fold dilution microtitre plate method, in the minimal liquid yeast nitrogen base (YNB) medium without amino acids and ammonium sulphate containing 2% glucose and L-proline (4 mg/mL). Wells containing serially diluted test compounds and control were inoculated with 10<sup>4</sup> cells/mL of an overnight culture of fungal cells and the microtitre plates were incubated for 24 h at 30°C. Fungal growth was measured using the microplate reader (Labsystem Multiscan Biochromatic) Please provide city and state for Labsystem Multiscan Biochromatic at λ = 595 nm. The MIC was defined as the inhibitor concentration preventing at least 80% of fungal growth, as compared with the inhibitor-free control.

## Results and discussion

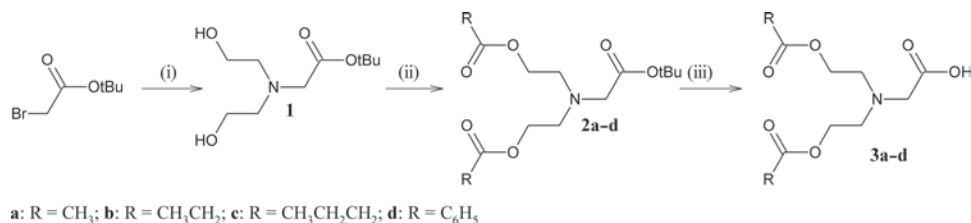
### Chemistry

The syntheses of compounds **3a–d** (Scheme 1) was started with preparation of tert-butyl ester of bis-*N,N*-(2-hydroxyethyl)glycine (**1**). Compound **1** was prepared by a displacement reaction using tert-butyl bromoacetate. Condensation of compound **1** with corresponding carboxylic acids was carried out in the presence of DCC/DMAP<sup>17</sup> to give appropriate tert-butyl ester of bis-*N,N*-(2-acyloxyethyl)glycine (**2a–d**). Deprotection of the carboxyl group was performed in anhydrous trifluoroacetic acid. The bicine derivatives **3a–d** were prepared as trifluoroacetate salts. In the case of the FMDP acyloxyalkyl esters synthesis (Scheme 2), BocFMDP (**4**) was treated with an appropriate chloroalkyl esters<sup>18</sup> in the presence of DBU and NaI.<sup>19</sup> The reaction proceeds smoothly in a polar solvent such as acetonitrile. The products were purified by silica gel column chromatography with ethyl acetate:petroleum ether (1:2) as a mobile phase. After deprotection of the amino group using anhydrous TFA in CH<sub>2</sub>Cl<sub>2</sub>, compounds **6a–f** were obtained as hygroscopic trifluoroacetate salts. The synthesis of compounds **8a–i** was performed using standard synthetic procedures

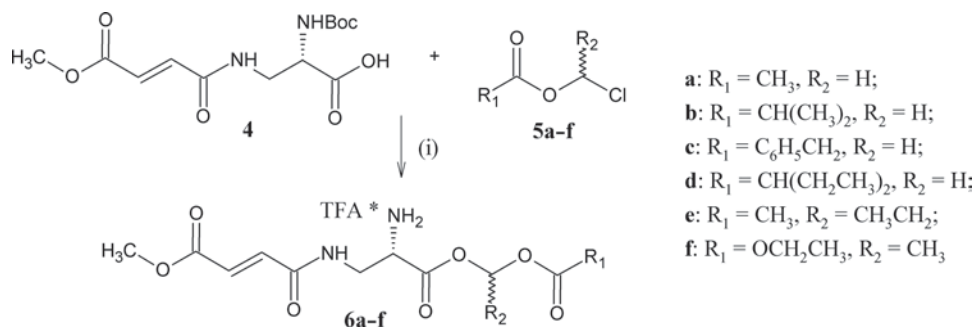
(Scheme 3). The compounds **3a-d** were activated with *N*-hydroxysuccinimide (HOSu) and DCC<sup>20</sup> to give the active esters (**7a-d**) and condensed with appropriate acyloxyalkyl esters of FMDP (**5a-f**). The products were purified by silica gel column chromatography with ethyl acetate:petroleum ether (3:1) as a mobile phase. Using anhydrous hydrochloride in diethyl ether, compounds **8a-i** were obtained as amorphous hydrochloride salts.

### Membrane affinity

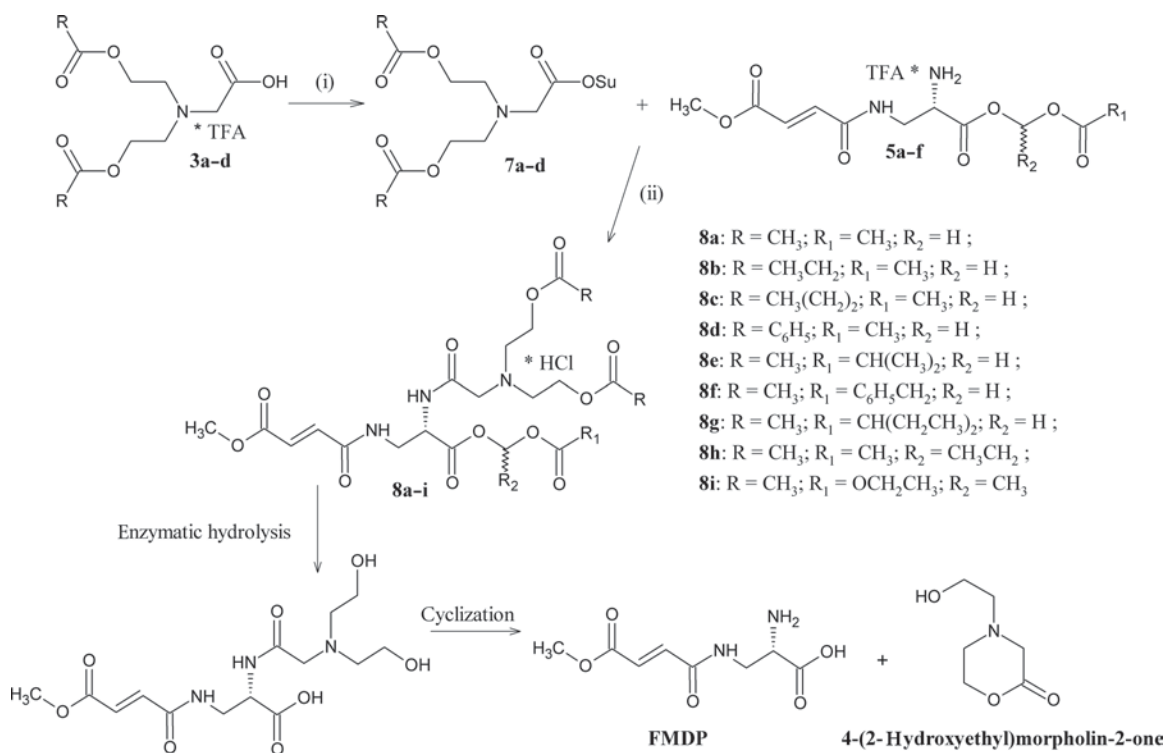
The affinity of a drug for biological membrane is an important factor facilitating its diffusion into fungal cell. For compounds **8a-i**, their interaction with a biological membrane model was investigated using an HPLC chromatographic column immobilized artificial membrane (IAM) PC.DD 2 with stationary phase mimicking the cell membrane. The retention



Scheme 1. Syntheses of bis-*N,N*-(2-acyloxyethyl)glycine trifluoroacetate (**3a-d**). (i) diethanolamine, CH<sub>2</sub>Cl<sub>2</sub>; (ii) RCOOH, DMAP, DCC and (iii) TFA.



Scheme 2. Syntheses of FMDP acyloxyalkyl esters. (i) CH<sub>3</sub>CN, DBU, NaI. \* denotes salt formation.



Scheme 3. Syntheses of acyloxyalkyl ester of *N*<sup>2</sup>-(bis-*N,N*-(2-acyloxyethyl)glyciny)-*N*<sup>3</sup>-(4-methoxyfumaroyl)-(L)-2,3-diaminopropanoic acid hydrochloride (**8a-i**), (i) HOSu/DCC and (ii) HCl/Et<sub>2</sub>O and hypothetical degradation pathway. \* denotes salt formation.

Table 1. Antifungal activity of compounds **8a-i** and their affinity to biological membrane.

Compound	R	R <sub>1</sub>	R <sub>2</sub>	MIC (µg/mL)				log <i>k'</i> <sub>IAM,0</sub>
				<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>	<i>S. cerevisiae</i>	
<b>8a</b>	CH <sub>3</sub>	CH <sub>3</sub>	H	500	500	1000	1000	0.91
<b>8b</b>	CH <sub>3</sub> CH <sub>2</sub>	CH <sub>3</sub>	H	1000	1000	500	1000	1.01
<b>8c</b>	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	H	1000	500	250	1000	1.36
<b>8d</b>	C <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	H	500	125	500	500	2.11
<b>8e</b>	CH <sub>3</sub>	CH(CH <sub>3</sub> ) <sub>2</sub>	H	125	125	62.5	31.3	1.34
<b>8f</b>	CH <sub>3</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	31.3	125	62.5	125	1.76
<b>8g</b>	CH <sub>3</sub>	CH(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	H	31.3	250	125	500	1.72
<b>8h</b>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub> CH <sub>2</sub>	62.5	125	125	250	1.55
<b>8i</b>	CH <sub>3</sub>	OCH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	125	250	15.6	125	1.26
FMDP	—	—	—	>2000	>2000	>2000	>2000	-0.85

time determined for the examined compounds was expressed as log*k'*<sub>IAM</sub> and extrapolated to log*k'*<sub>IAM,0</sub>. The highest log*k'*<sub>IAM,0</sub> value was determined for compound **8d** (containing an aromatic and methyl substituents), whereas the lowest one was determined for compound **8a** (with only methyl substituents). These values comprise not only the lipophilic properties but also other interactions with membrane such as hydrogen bond formation or electrostatic interactions. Therefore, the log*k'*<sub>IAM,0</sub> values reflect rather membrane affinity than lipophilic properties.

### Antifungal activity

MICs were determined in YNB medium with proline as carbon source. Table 1 present activity against few species. Most of the synthesized compounds exhibited moderate antifungal activity in the range from 15.6 to 1000 µg/mL and their activity is not correlated with apparent lipophilicity. The lack of correlation between the log*k'*<sub>IAM,0</sub> and MIC values may be attributed to the slow releasing of FMDP molecule from bicine conjugate. Compounds **8a-d** displayed almost the same antifungal activity thus showing that the type of substituent in "R" position is not important for the activity. On the other hand, the change of latent ester of FMDP in compounds **8e-i** (position "R<sub>1</sub>" and "R<sub>2</sub>") improves antifungal activity of these compounds. This may be explained by different rates of intracellular hydrolysis of these compounds resulting in higher level of FMDP inside the cells. All the obtained compounds have better lipophilic properties than free inhibitor—FMDP. The log*k'*<sub>IAM,0</sub> values between 0.91 and 2.11 show that compounds **8a-i** may be able to cross the cell membrane by free diffusion.

### Conclusion

We have synthesized a series of derivatives of bicine and glucosamine-6-phosphate synthase inhibitor. The modification of FMDP at the amino group improves lipophilic properties of obtained compounds. Compounds **8a-i** are soluble in water and show better antifungal activity than the parent compound—FMDP.

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### Declaration of interest

The authors report no conflicts of interest.

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