RAPID COMMUNICATION

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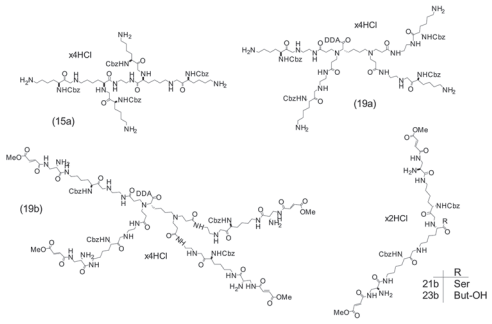

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RAPID COMMUNICATION

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Peptide dendrimers as antifungal agents and carriers for potential antifungal agent—N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diaminopropanoic acid—synthesis and antimicrobial activity

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A series of peptide dendrimers and their conjugates with antimicrobial agent FMDP (N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diamino-propanoic acid) were synthesized. The obtained compounds were tested for the antibacterial and antifungal activity. All novel dendrimers displayed much better activity against the tested strains than FMDP itself. Moreover, their conjugates with FMDP also exhibited antimicrobial activity. The most promising molecules were tested against a broad selection of fungal strains. The analysis of their antifungal properties indicates that the examined molecules are efficient growth inhibitors of fluconazole-resistant hospital-acquired strains. Moreover, an application of amphiphilic branched peptides such as FMDP carriers suggests that transport mechanism involves more likely the cell membrane perturbation than the mediation of the specific transport proteins. The activity of obtained compounds strongly depends on the specific structure of the molecule.

KEYWORDS

antimicrobial activity, drug delivery, FMDP, peptide dendrimers

1 | INTRODUCTION

An increasing number of fungal infections is a serious problem in today's world.[1] Candidiasis is considered to be one of the most difficult chronic diseases to be treated effectively. Systemic fungal infections are particularly dangerous for affected patients. Nowadays, the commonly occurring phenomenon of antibiotics abuse, the body sterilization, and immunodeficiency diseases are the main factors causing the increased number of severe and difficult to cure cases of candidiasis.[2] Moreover, there is a limited range of available antifungal drugs on the market; however, many of them, such as amphotericin B, are highly toxic and display a number of side effects. Therefore, there is an explicit need for new antifungals that would lead to effective therapy.[3,4]

The differences in the structures between the fungal and mammalian cells must be taken into account before selecting a specific molecular target in drug design. An example of such target is a cellular membrane, whose structure varies depending on organism (ergosterol for fungi and cholesterol for mammals).[4,5] Due to these differences, it is possible to develop a selective antifungal drug.[6] As another potential target for the design of antifungal agents, we proposed glucosamine-6-phosphate (GlcN-6-P) synthase, which catalyzes the first step in the biosynthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), a precursor of a number of aminosugars containing macromolecules including chitin and mannoproteins in fungi.[7]

The N^3-(4-methoxyfumaroyl)-(*S*)-2,3-diamino-propanoic acid (FMDP), the most selective and effective inhibitor of GlcN-6-P synthase,[8] was proposed as an antifungal agent. Although this compound has shown the high activity against the pure enzyme, it displayed, however, only a very poor antimicrobial activity. This effect is likely caused by the high polarity of the inhibitor molecule which prevents its effective penetration through the cell wall.[8] The modifications of the inhibitor molecule by increasing its lipophilicity,[9,10] synthesis of latent ester derivatives,[11,12] and FMDP incorporation into short

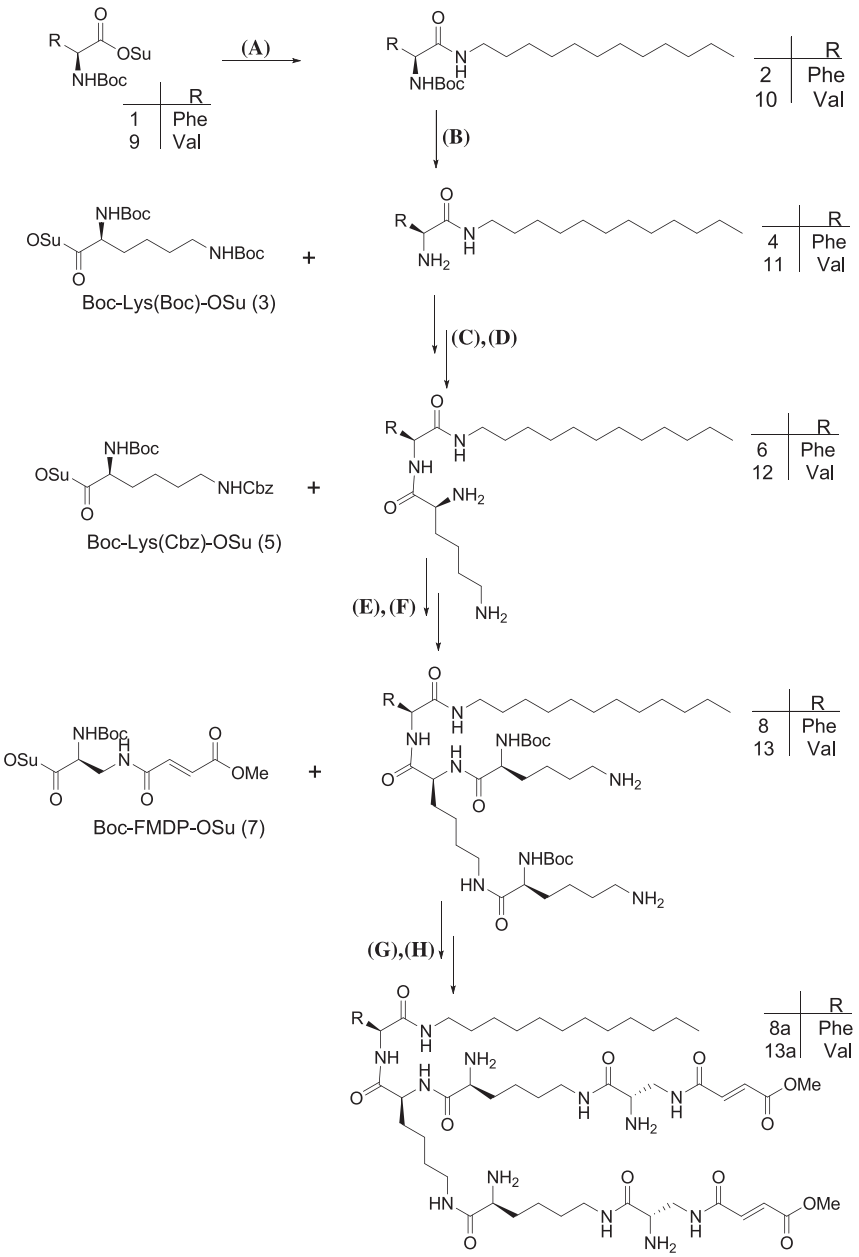
peptides were also tested.[13] However, none of these approaches has so far provided the expected results.

Therefore, the development of suitable FMDP carrier, a molecule capable of transporting active compound to its site of action and releasing it there, might solve the problem. For this purpose, we proposed to design a group of polyfunctional, amphiphilic peptide dendrimers that are carrying multiple positive charges and two or four FMDP residues located at terminal positions. Dendrimers, in general, are a vast family of nanosized cascade molecules with branched structure and multiple chemical functionalities on the surface. Due to their properties, dendrimers such as polyamidoamine (PAMAM's) or poly (amidoamine-organosilicone) (PAMAMOS) and other constructed from amino acids (Lys, Orn, and Arg) are widely used in many applications, including drug delivery. Interestingly, dendrimers themselves may also be used as antimicrobial agents. There are many literature reports on the possibility of using them as drug carriers in pharmacology and in gene therapy [14-21]or as an antimicrobial agents by themselves.[22]

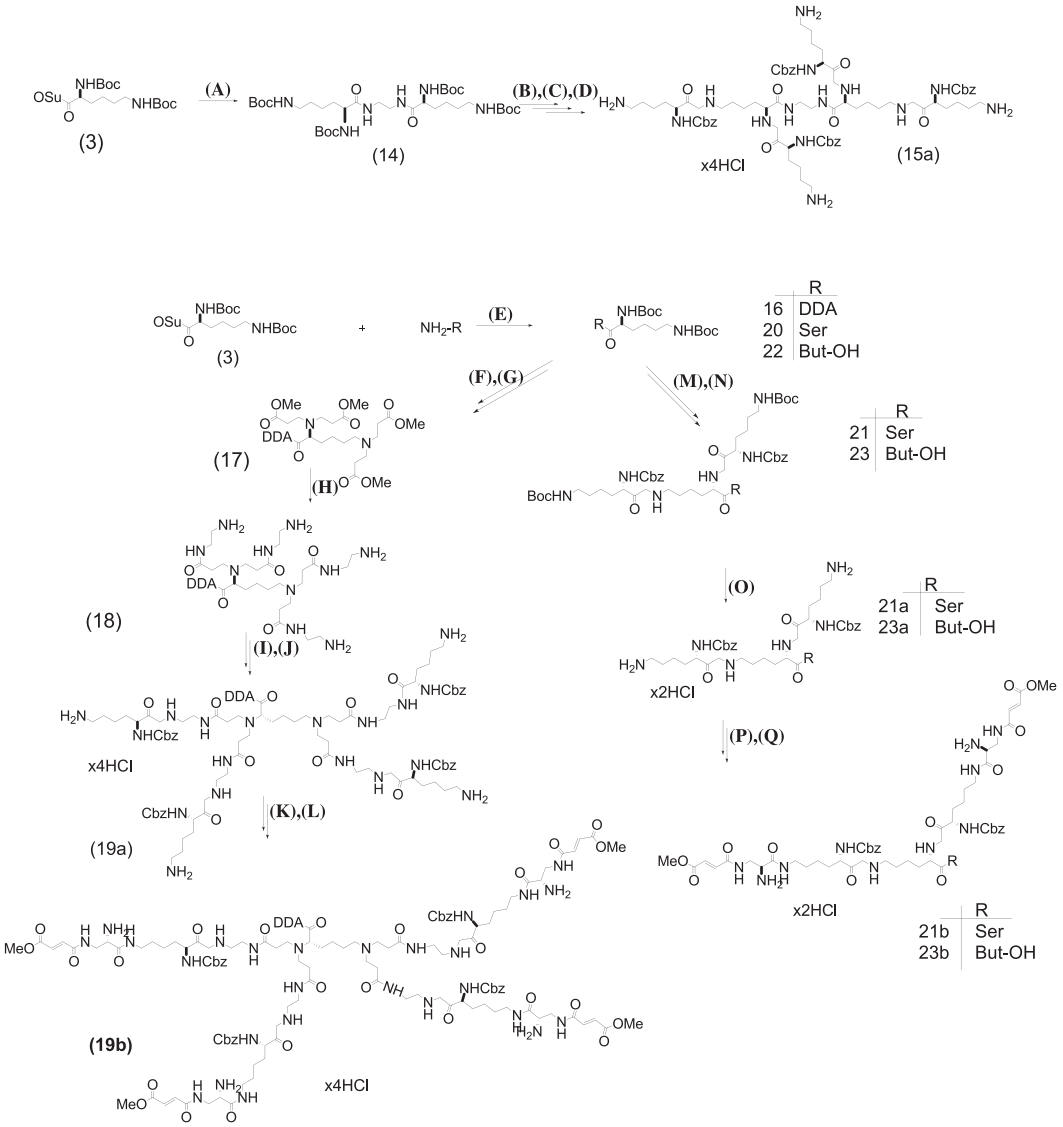
In this communication, we report the synthesis of novel Lys-based peptide dendrimers and their conjugates with FMDP and evaluation of their antimicrobial and antifungal activity.

2 | SYNTHESIS OF PEPTIDE DENDRIMERS

The synthesis of peptide dendrimers (Schemes 1 and 2) was carried out by the divergent method by the active esters, giving oily products in acceptable yields. Obtained cascade molecules were used as a transport system for the selective GlcN-6-P synthase inhibitor, ie, FMDP molecule, which was obtained by the known procedure,[8] or alone as active agents themselves. Deprotection of the Boc groups



SCHEME 1 Synthesis of dendrimers **8** and **13** and their conjugates with FMDP. (A) dodecylamine (DDA), THF, rt, 24 h, yield: 90% (2), 89% (10); (B) HCl/dioxane, rt, 4 h, yield: 92% (4), 90% (11); (C) MeOH/water, rt, 24 h; (D) HCl/dioxane, rt, 4 h, yield: 90% (6), 90% (12); (E) MeOH/water, rt, 24 h; (F) H_2, Pd/C, rt, 4 h, yield: 72% (8), 60% (13); (G) MeOH/water, rt, 24 h; (H) HCl/dioxane, rt, 4 h, yield: 69% (**8a**), 77% (**13a**)



SCHEME 2 Synthesis of peptide dendrimers **14-23** and their conjugates with FMDP. (A) MeOH/water, rt, 24 h, yield: 62%; (B) HCl/dioxane, rt, 4 h; (C) Cbz-Lys (Boc)-OSu, MeOH, rt, 24 h yield: 63%; (D) HCl/dioxane, rt, 4 h yield: 86%; (E) Ser, DDA or But-OH, MeOH/water, rt, 24 h, yield: 67% (16), 69% (20), 89% (22); (F) HCl/dioxane, rt, 4 h; (G) methyl acrylate (MA), MeOH/water, rt, 24 h, yield: 53%; (H) EDA, MeOH/water, rt, 24 h, yield: 65%; (I) Cbz-Lys (Boc)-OSu, MeOH, rt, 24 h yield: 62%; (J) HCl/dioxane, rt, 4 h yield: 86%; (K) Boc-FMDP-OSu, MeOH/water, rt, 24 h, (L) HCl/dioxane, rt, 4 h yield: 85%; (M) HCl/dioxane, rt, 4 h; (N) Cbz-Lys (Boc)-OSu, MeOH, rt, 24 h yield: 69% (21), 68% (23); (O) HCl/dioxane, rt, 4 h yield: 88% (**21a**), 93% (**23a**); (P) Boc-FMDP-OSu (7), MeOH/water, rt, 24 h; (Q) HCl/dioxane, rt, 4 h yield: 86% (**21b**), 81% (**23b**)

was carried out with 4M HCl in dioxane, providing products as amorphous hydrochloride salts in a good yield, while deprotection of Cbz group was carried out by catalytic hydrogenation in the presence of a catalyst (5% Pd/C) also providing products in a good yield. In the case of compounds P15-P23 and their derivatives, the presence of Cbz protective group was required to improve the hydrophobicity of the compounds, thus increasing their activity. As assumed, the removal of the Cbz group resulted in the loss of these molecules' antimicrobial activity.

New compounds were characterized by NMR and mass spectrum analysis and their structures confirmed. Both free dendrimers and their conjugates with FMDP were tested for the minimum inhibitory concentration (MIC) against Gram-positive and Gram-negative bacteria as well as for *Candida spp*. grown in two different culture media. Obtained results are presented in Tables 1 and 2.

3 | BIOLOGICAL ACTIVITY

Data presented in Table 1 indicate that some of the new compounds showed moderate potency against *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* reference strains, with compounds **8a** and **13a** bearing FDMP residue being the most broadly active. The most active against *C. albicans* ATCC 10231 was amphiphilic compound **19a**, with four flexible arms terminated solely with amine groups. Moreover, all compounds displayed much better activity against

TABLE 1 Minimal inhibitory concentration (MIC) of the synthesized compounds and FMDP (μM)

		Substituents			MIC, μM		
					RPMI 1640	MHB	MHB
Compound	Charge	R	α-Lys arm	ε-Lys arm	*Candida albicans* ATCC 10231	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25925
8a	+4	CH_2-Ar	NH-FMDP	NH_3^+	49	25	25
13a	+4	CH $(CH_3)_2$	NH-FMDP	NH_3^+	94	26	26
15a	+4	———	NH-Cbz	NH_3^+	79	159	39
18a	+4	NH-FMDP	———	———	585	585	293
19a	+4	———	NH-Cbz	NH_3^+	15	249	31
19b	+4	———	NH-Cbz	NH-FMDP	357	357	357
21a	+2	Ser	NH-Cbz	NH_3^+	145	>100	145
23a	+2	But-OH	NH-Cbz	NH_3^+	150	120	300
FMDP	+1	———	———	———	>500	>500	>500

tested strains than FMDP itself. The FMDP-functionalized branched peptides **8a** and **13a** were widely tested against the set of reference, hospital-acquired, and genetically modified *Candida spp*. strains grown in two culture media. Fluconazole was used as a reference compound (Table 2). The results of the experiment showed that the tested compounds exhibited higher antifungal activity when grown in YNB-SA minimal medium than in rich, simulating physiological conditions RPMI medium.

TABLE 2 Antifungal potency of dendrimers **8a** and **13a** against *Candida spp*. measured as minimal inhibitory concentration (MIC) in two culture media

	MIC, μM					
	YNB-SA			RPMI 1640		
Strain	8a	13a	Fluconazole	8a	13a	Fluconazole
Candida albicans ATCC 10231	22	40	5.7	49	94	38
Candida albicans S. C5314	33	36	2.7	75	100	5.6
Candida albicans OPT opt1-opt5Δptr2Δptr22Δ	24	47	3.1	97	>100	25
Candida albicans B3	36	32	5.7	80	>100	88
Candida albicans B4	27	29	75	72	>100	>400
Candida albicans Gu4	34	37	3.1	79	89	12
Candida albicans Gu5	22	38	>400	92	98	>400
Staphylococcus cerevisiae	11	19	22	22	26	56
Candida glabrata DSM 11226	17	44	38	90	97	50
Candida krusei DSM 6128	18	41	350	86	69	400
Candida parapsilosis DSM 5784	45	60	——	73	>100	——

Collection of used *Candida* species included three wild strains, two hospital-acquired fluconazole-sensitive strains (*C. albicans* B3- and *C. albicans* Gu4-) isolated from AIDS patients prior to initiation of antifungal therapy, and two strains overproducing Cdr1p and Cdr2p (*C. albicans* Gu5) or Mdr1p (*C. albicans* B4) pumps isolated from the same patients after long-term treatment with fluconazole.[23] It should be emphasized that both dendrimers **8a** and **13a** efficiently inhibited the growth of both fluconazole-insensitive strains grown in RPMI medium.

Usually, peptides are mostly taken up by microorganisms *via* peptide permeases. In our study analysis of results obtained for the *C. albicans* SC5314 and its mutant *C. albicans OPT* indicated that the transport of active compounds **8a** and **13a** may not be mediated by peptide permease. These strains with knocked permease genes are devoid of peptide permeases. Broad activity of dendrimers **8a** and **13a,** as well as a high antifungal potency of dendrimer **18a,** suggests that permeation of cell membrane by cationic dendrimers might be a route of transport of the tested dendrimers into the cells. The hydrophobic dodecylamine (DDA) chains present in the dendrimeric structures may be incorporated into the microorganism membranes. More conclusions could be drawn from experiments in which N-acetylglucosamine (NAG), a natural inhibitor of GlcN-6-P synthase, was added to YNB-SA medium. In case the enzyme GlcN-6-P synthase was the only target for the tested **8a** and **13a** dendrimers, the addition of NAG would result in the complete prevention of anticandidal activity. However, for the tested compounds, no such an effect was observed, which suggests that GlcN-6-P synthase is not the only target for the new conjugates. It is important to point out that compounds **8a** and **13a** showed activity also against fluconazole-resistant strain *C. krusei*.

Fungal strains used to test antimicrobial activity:

• *C. albicans* OPT *opt1-opt5Δptr2Δptr22Δ*—Mutant of *C. albicans* SC 5314 lacking peptide permeases,

• *C. albicans* SC5314—Wild strain, Collection of Gdansk University of Technology,

• *C. albicans* Gu4—Fluconazole-sensitive, isolated from AIDS patient prior to initiation of therapy, initial for Gu5, University of Wurzburg

• *C. albicans* Gu5—Isolated after long-term treatment with fluconazole, overproducing Cdr1p and Cdr2p

• *C. albicans* ATCC 10231—Wild strain, American Collection

• *C. albicans* B3—sensitive to fluconazole, isolated from AIDS patients prior to initiation of therapy, initial for B4, University of Wurzburg

• *C. albicans* B4—Isolated from patient after long-term treatment with fluconazole, University of Wurzburg,

• *S. cerevisiae* ATCC 9763—Wild strain, American Collection

• *C. glabrata* DSM 11226—Wild strain, Collection of DSMZ,

• *C. krusei* DSM 6128—Wild strain, Collection of DSMZ,

• *C. parapsilosis* DSM 5784—Wild strain, Collection of DSMZ, Cdr1p and Cdr2p pumps, University of Wurzburg.

4 | CHEMISTRY

Thin layer chromatography (TLC) was performed on aluminum plates (Kieselgel 60 F254, Merck) and compounds visualized by ultraviolet (UV) light and crystalline iodine vapors (POCH). Adsorption column chromatography was carried out using silica gel (70-230 mesh, Merck), while the exclusion chromatography with a Sephadex LH-20, Pharmacia Fine Chemicals. The MS spectra were recorded using Agilent Technologies 6540 UDH Accurate-Mass Q-TOF and the NMR spectra were recorded on Gemini Varian spectrometer operating at 500 MHz using DMSO and D2O as solvents.

4.1 | General procedure for the preparation of peptide dendrimers and conjugates

Peptide dendrimers were obtained using active esters method as shown in Schemes 1 and 2. The substrate with free amino groups, eg, [18], was dissolved in methanol, then 50% excess of corresponding active ester in methanol was added successively. Reaction was carried out at room temperature, intensively mixed for 3 to 5 days, progress controlled by TLC, then the solvent was evaporated to dryness and the postreaction mixture was dissolved in methanol. The compounds were purified on Sephadex LH-20 with methanol as mobile phase, then the solvent was removed in vacuo to give the title derivatives.

Conjugates were obtained analogously using active esters method. Peptide dendrimers with free amino groups were dissolved in methanol, then 50% excess of Boc-FMDP active ester in methanol was added successively. After the reaction was completed (5 days), solvent was evaporated, and the product was purified on a Sephadex LH-20 column in methanol. The column was washed with methanol, and fractions containing the title compound were collected and evaporated to give conjugates as the light yellow oil.

4.2 | General method for the Boc deprotection

The *tert*-butoxycarbonyl protecting group was removed by treating compounds with 4M HCl in dioxane (5 mL) for 4 hours, followed by removing the solvent in vacuo. The products were triturated with diethyl ether and after filtration, and the precipitate was dried in vacuo over KOH.

4.3 | General method for the Cbz deprotection

The carboxybenzoxy protective group was removed by catalytic hydrogenation of the compound dissolved in methanol in the presence of a catalyst (5% Pd/C) followed by removing the solvent in vacuo. The products were triturated with diethyl ether and were filtered off. The precipitates were dried in vacuo over KOH.

(8a) $C_{55}H_{96}N_{12}O_{12}$*4HCl, M.W. 1259,24

MS (ESI, MeOH): m/z: 1136$[M + Na]^+$

^{1}H NMR (500 MHz, D_2O), δ: 0.85 (m, 3H, **CH_3**-DDA), 1.2-1.8 (m, 76H, **β, γ, δ**-Lys, **$C^{2\text{-}11}$**-DDA), 2.7-3.1 (m, 20H, **ε**-Lys, **β**-Phe, **C^1**-DDA), 2.85-3.0 (m, 4H, CCH**CH_2**-FMDP), 3.9-4.5 (m, 6H, **α**-Lys, **α**-Phe, **α**-FMDP), 6.7-7.3 (m, 4H, **CH**=**CH**-FMDP), 7.15-7.6 (m, 5H, **Ar**-Phe)

(13a) $C_{51}H_{96}N_{12}O_{12}$*4HCl, M.W. 1211,19

MS (ESI, H_2O): m/z: 1066$[M + H]^+$

^{1}H NMR (500 MHz, D_2O), δ: 0.7-0.9 (m, 9H, **CH_3**-DDA, **CH_3**-Val), 1.3-2.0 (m, 77H, **β, γ, δ**-Lys, **$C^{2\text{-}11}$**-DDA, **β**-Val), 2.8-3.2 (m, 18H, **ε**-Lys, **C^1**-DDA), 2.8-3.1 (m, 4H, CCH**CH_2**-FMDP), 3.8-4.5 (m, 6H, **α**-Lys, **α**-Val, **α**-FMDP), 6.5-7.0 (m, 4H, **CH**=**CH**-FMDP)

(15a) $C_{74}H_{116}N_{14}O_{14}$*4HCl, M.W. 1567,61

MS (ESI, H_2O): m/z: 734$[M + 2Na]^{2+}$, 1444$[M + Na]^+$

^{1}H NMR (500 MHz, D_2O), δ: 1.2 (s,12H, **γ**-Lys), 1.4(m, 12H, **δ**-Lys), 1.7 (m, 12H, **β**-Lys), 2.2 (m, 12H, **ε**-Lys), 3.1-3.2 (m, 4H, NH**CH_2CH_2**NH), 3.7-4.3(m, 6H, **α**-Lys), 5.3 (ss, 28H, **CH_2**-Ar, **Ar**)

(18a) $C_{70}H_{123}N_{19}O_{21}$*4HCl, M.W. 1708,65

MS (ESI, H_2O): m/z: 544$[M + 3Na]^{3+}$, 804,5$[M + 2Na]^{2+}$, 1564$[M + H]^+$

^{1}H NMR (500 MHz, DMSO), δ: 0.84(**C^{12}**-DDA), 1.23(**C^{11}**-DDA), 1.25(**C^6**-DDA), 1.31(**C^3**-DDA), 1.43(**C^{10}**-DDA), 1.68(**C^5**-DDA), 1.69 (**C^2**-DDA), 1.79(**C^4**-DDA), 2.3-2.8(8H, NH**CH_2**CO-FMDP), 2.77(**C^8**-DDA), 2.85-3.65(32H, N**CH_2CH_2**CO, NH**CH_2CH_2**NH), 3.0-3.6(8H, **β, γ, δ, ε**-Lys), 3.01(**C^7**-DDA), 3.12(**C^9**-DDA), 3.59(**C^1**-DDA), 3.6(**α**-Lys), 3.63(12H, **OMe**), 3.65(4H, **α**-FMDP), 6.86(8H, **CH**=**CH**), 7.9-9.2(12H, **NH**CO), 8.45(4H, **NH^+**-FMDP), 10.17(**NH**-C^1-DDA)

^{13}C NMR (500 MHz, DMSO), δ: 14.4(**C^{12}**-DDA), 22.5(**C^{11}**-DDA), 25.0-32.0(4C, NH**CH_2**CO-FMDP), 25.1(**C^5**-DDA), 25.2(**C^2**-DDA), 26.5 (**C^6**-DDA), 29.1(**C^{10}**-DDA), 30.5(**C^8**-DDA), 32.6(**C^3**-DDA), 32.8(**C^4**-DDA), 33.0-48.0(16C, N**CH_2CH_2**CO, NH**CH_2CH_2**NH), 36.0-46.0(9C, **α, β, γ, δ, ε**-Lys, FMDP), 38.9(**C^9**-DDA), 40.0(**C^1**-DDA), 45.9(**C^7**-

DDA), 132.8(8C, **CH**=**CH**), 165.0(4C, NH**CO**CH=CH), 169.0-174.5(8C, **NH**CO-MA, **NH**CO-MA), 170.9(4C, **CO**OMe), 172.1(**CO**-C^1-DDA)

(19a) $C_{98}H_{158}N_{18}O_{17}$*4HCl, M.W. 2006,25

MS (ESI, H_2O): m/z: 981[M + 2H]$^{2+}$, 953[M + 2Na]$^{2+}$, 1861[M + H]$^+$

^{1}H NMR (500 MHz, DMSO), δ: 0.85 (m, 3H, **CH_3**-DDA), 1.3-1.4 (m, 82H, C **$(CH_3)_3$,γ**-Lys), 1.45-2.0 (m, 40H, **β, δ**-Lys, **C^{2-11}**-DDA), 2.5-2.9 (m, 13H, **ε**-Lys, **C^1**-DDA), 3.0-3.8 (m, 32H, NH**CH_2CH_2**NH, N**CH_2CH_2**CO), 3.85-4.1 (m, 5H, **α**-Lys), 6.5-7.2 (m, 13H, **NH**CO), 7.1-8.0 (m, 12 H, **NH**CO)

(19b) $C_{130}H_{198}N_{26}O_{33}$*4HCl, M.W. 2798,96

MS (ESI, H_2O): m/z: 885[M + 3H]$^{3+}$, 907[M + 3Na]$^{3+}$, 1349[M + 2Na]$^{2+}$

^{1}H NMR (500 MHz, DMSO), δ: 0.85 (m, 3H, **CH_3**-DDA), 1.3-1.4 (m, 82H, C **$(CH_3)_3$,γ**-Lys), 1.45-2.0 (m, 40H, **β, δ**-Lys, **C^{2-11}**-DDA), 2.5-2.9 (m, 13H, **ε**-Lys, **C^1**-DDA), 3.0-3.8 (m, 52H, CCH**CH_2**-FMDP, NH**CH_2CH_2**NH, N**CH_2CH_2**CO, O**CH_3**-FMDP), 3.85-4.1 (m, 9H, **α**-Lys, **α**-FMDP), 6.5-7.2 (m, 21H, **CH**=**CH**-FMDP, **NH**CO), 7.1-8.0 (m, 12 H, **NH**CO)

(21a) $C_{38}H_{59}N_7O_8$*2HCl, M.W. 858,85

MS (ESI, H_2O): m/z: 787[M + H]$^+$

^{1}H NMR (500 MHz, D_2O), δ: 1.2 (m, 6H, **γ**-Lys), 1.4 (m, 6H, **δ**-Lys), 1.7-1.9 (m, 6H, **β**-Lys), 2.4 (m, 6H, **ε**-Lys), 3.0 (s, 1H, NHCHCH_2**OH**-Ser), 3.4 (m. 2H, NHCH**CH_2**OH-Ser), 3.6 (s, 4H, **CH_2**-Ar), 3.75-4.25 (m, 4H, **α**-Lys, **α**-Ser), 4.7 (m, 10H, **Ar**), 6.6-6.9 (m, 1H, **COOH**-Ser)

(23a) $C_{39}H_{63}N_7O_8$*2HCl, M.W. 828,87

MS (ESI, H_2O): m/z: 379[M + 2H]$^{2+}$, 757[M + H]$^+$,

^{1}H NMR (500 MHz, DMSO), δ: 1.2 (m, 6H, **γ**-Lys), 1.3-1.9 (m, 18H, **β, δ**-Lys, **C^{2-4}**-But), 2.2-2.5 (m, 6H, **ε**-Lys), 3.0-3.8 (m, 2H, **C^1**-But), 3.9-4.5 (m, 4H, **α**-Lys, **OH**-But), 5.4 (s, 14H, **Ar**)

4.4 | In vitro antimicrobial activity

Minimal inhibitory concentrations (MICs) for fungal strains were determined four times by the serial twofold microdilution method in YNB medium with ammonium sulfate as a nitrogen source at pH 5.0 as described before[10] and in RPMI 1640 medium (pH 7.0), according to the CLSI recommendations (M27-A3 document, Clinical Laboratory Standards Institute 2008).[23] Analogous tests were provided for bacterial strains, using Mueller Hinton Broth 2 medium at pH 7.3, followed by procedure described in the CLSI M07-A10 document.[24,25] Optical cell density was measured using the microplate reader Victor3, Perkin Elmer. The MIC value was defined as the minimal concentration of compound inhibiting the growth of the cells in comparison with the positive control. Obtained results were compared with the control molecule, FMDP. Tests were performed on both wild and clinical strains.

5 | CONCLUSION

A series of peptide dendrimers and their conjugates with an antifungal agent FMDP were synthesized and tested. All compounds displayed much better activity against the tested strains than FMDP itself. The most promising molecules were tested against a broad selection of fungal strains. The analysis of their antifungal properties indicates that the examined molecules are efficient growth inhibitors of fluconazole-resistant hospital-acquired strains. Moreover, application of amphiphilic branched peptides such as FMDP carriers suggests that transport mechanism involves more likely the cell membrane perturbation than the mediation of the specific transport proteins.

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