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Amide derivatives of partricin A with potent antifungal activity

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Summary — A series of partricin A amides were synthesized using the active ester method by reaction with several amines on the carboxy group and then with some acids on the mycosamine group of partricin A. Most of the derivatives are more potent antifungals than the known reference standards, including amphotericin B, and a few are also less toxic and less hemolytic. Amides substituted with basic groups may give hydrosoluble salts, useful for injectable formulations, and two derivatives were selected for further development, namely, partricin A 2-dimethylaminoethyl amide (**10**, SPA-S-710) and *N*-dimethylaminoacetyl-partricin A 2-dimethylaminoethyl amide (**22**, SPA-S-752).

partricin A amide / polyene antibiotic / antifungal activity/ structure–activity relationship

Introduction

Several years ago we isolated a polyene antibiotic referred to as partricin [1] from the fermentation broths of *Streptomyces aureofaciens* NRRL 3878. This antibiotic was endowed with high antifungal activity (particularly against *Candida albicans*) and antiprotozoal activity, but it was thought to be too toxic for clinical use.

In an effort to improve its biological properties, some derivatives of partricin were prepared, including partricin methyl ester (USAN mepartricin). This proved to be the first successful semisynthetic polyene, being more effective and less toxic than the parent compound [2, 3].

Additional studies demonstrated that partricin and its derivatives were constituted of two components A and B: partricin A (fig 1) and partricin B without the *N*-methyl substituent on the aromatic amino group. The complete structural formula of the two compounds was elucidated and their biological activities were found to be very similar [4, 5].

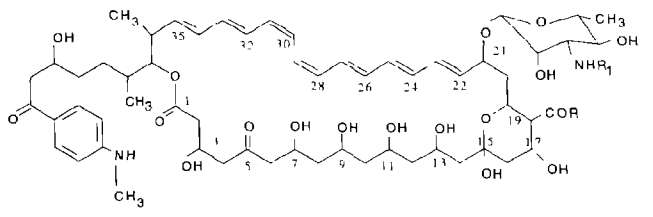


Fig 1. Structural formula of partricin A (R = OH, R_1 = H) and its amide derivatives (R and R_1, see table I)

Fermentation of a new mutant strain of *S aureofaciens* has led to partricin A that is almost free from the B component. We decided to prepare a new series of amide derivatives, using this single compound as a starting material, in order to improve their chemical and biological characterization.

Chemistry

The new amide derivatives are substituted with amino groups on the carboxy group of partricin A and may be substituted with acyl groups on the mycosamine amino group, thus giving diamide derivatives (fig 1). Both the amino and the acyl substituents may carry basic groups, in agreement with structure–activity relationship data reported for a few derivatives of amphotericin B and other polyenes [6–8]. Basic substituents may also be useful for preparation of hydrosoluble salts, which are useful for injectable formulations.

The synthesis of the new compounds has been carried out by the active ester method, using diphenyl phosphorazidate as the activating agent of the carboxy groups and dimethylacetamide as the solvent [9]. The course of the reaction was followed by means of thin-layer chromatography on silica gel, while the products were purified by silica-gel column chromatography.

Purity was checked by HPLC analysis and structure was confirmed by elementary analysis, UV and IR spectroscopy and mass spectrometry, as needed. The compounds are yellow substances, which are almost insoluble in water and most organic solvents and soluble in dimethylsulfoxide and dimethylacetamide. When substituted with basic groups, they give salts with many organic and inorganic acids, which are soluble in water.

Biology

The antifungal activity was screened by determining the inhibitory concentrations against three strains of *C albicans* and one strain each of *Saccharomyces cerevisiae*, *Mucor* sp, *Aspergillus niger* and *Trichophyton mentagrophytes* and the antiprotozoal activity against one strain of *Trichomonas vaginalis*. Potent antimicrobial activity is defined as a strong affinity towards the microbial cellular target, ie, towards membrane ergosterol [10].

The toxicity of the products was checked first by measuring their hemolytic activity on rat erythrocytes in order to select derivatives with the lowest affinity to the red blood cell membrane cholesterol. Affinities that are similar for the microbial and animal cellular targets, ergosterol and cholesterol, respectively, are thought to be responsible for the poor selective toxicity of polyene macrolides [10]. The toxicity of the compounds was also checked by measuring the approximate LD_{50} in mice following intraperitoneal (or intravenous) administration.

For comparative purposes, all the tests were carried out against the starting polyene partricin A, the potent antifungal derivative mepartricin A and amphotericin B, which remains the most effective polyene antibiotic available for the treatment of deep-seated mycotic infections in humans [11].

Results and discussion

The structure of the synthesized derivatives is reported in table I, including secondary and tertiary amides (**1–9**), some of which were substituted with basic radicals (**10–21**), and diamides obtained by further substitution on the mycosamine aminogroup with basic acyl groups (**22–35**). The synthetic methods used are also indicated along with some characterizing data, such as the R_f value in TLC and RT in HPLC.

Antifungal and antiprotozoal data of all the compounds are reported in table II. The starting polyene partricin A **36**, mepartricin A **37** and amphotericin B **38** were used as reference compounds. The activity against *C albicans* was checked against three strains, in liquid and solid media after 24 and 48 h incubation times, and is expressed as a minimum inhibitory concentration (MIC, ng/mL). Almost all the derivatives were far more effective than amphotericin B **38** (MIC 240–480 ng/mL), most were more effective than partricin A **36** (MIC, 30–60 ng/mL). A few (**1, 10, 11**) were more effective (MIC 3.75–15 ng/mL) than mepartricin A itself **37** (MIC, 7.5–30 ng/mL) while others were equipotent (**2, 12–17**) to just a little less potent (**3, 20–22, 26–31**). The strong antifungal activity was evidenced for all the *C albicans* strains, in both the liquid and solid media; the activity was also maintained at 24 and 48 h control times, demonstrating the good stability of the compounds in the culture media.

The activity against *S cerevisiae* has been tested against one strain and one incubation time in liquid medium and is expressed as 50% inhibitory concentration (IC_{50}, ng/mL). A fair activity is shown by all the derivatives in the series and their potency is much higher than that of amphotericin B **38** (MIC, 35.5 ng/mL). With just a few exceptions (**5, 34, 35**), the activity is similar to that of partricin A **36** (MIC, 2.1 ng/mL) and mepartricin A (MIC, 1.0 ng/mL); the most effective compounds are **1, 10–15, 17, 20** and **21**, which showed MIC values lower than 1.0 ng/mL.

For filamentous fungi, MIC (μg/mL) evaluation against a *Mucor* sp strain showed that most of the compounds are much more effective than the starting partricin A **36** (MIC, 1.2 μg/mL) and had same or twice the potency of mepartricin A **37** (MIC, 0.6 μg/mL). A few of the compounds had the same (**19**) (MIC, 0.15 μg/mL) or half (**2, 3, 6–9, 11, 14, 15**) (MIC, 0.3 μg/mL) the effectiveness of amphotericin B **38**, which was the most potent compound against this single microorganism. For the whole series, the activity was not appreciably modified at the second control time (48 h).

Most derivatives were extremely potent against *A niger*, with much lower MIC values than the reference compounds, which were, in the order of increasing activity, partricin A **36**, amphotericin B **38** and mepartricin B **37**. With the exception of a few compounds with no (**34, 35**) or little activity, many derivatives (**2–4, 6, 12–15, 17, 20, 21**), showed MIC values of 0.075 μg/mL, while the others were in the range 0.15–0.3 μg/mL. Partricin A had an MIC of 2.4 μg/mL and amphotericin B 1.2 μg/mL; only mepartricin has a similar activity (0.15 μg/mL).

T mentagrophytes was also very sensitive to most of the derivatives, many of which (**10–13, 16, 17, 20, 22, 24, 25, 29**) were more effective than mepartricin (MIC values of 0.3 μg/mL and 0.6 μg/mL); other derivatives (**1, 14, 15, 21, 23, 26–28, 30–33**) were at least equipotent (MIC, 0.6 μg/mL) with mepartricin. The

Table I. Derivatives of patricin A (structural formula, see fig 1), hemolytic activity and acute toxicity in mice.

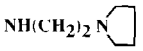
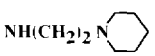
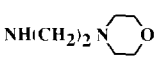
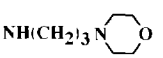
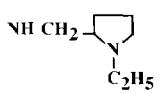
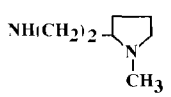
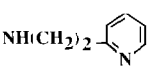
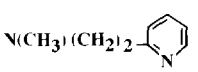
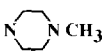
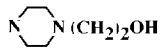
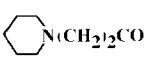
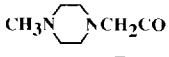
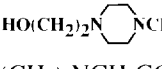
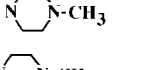
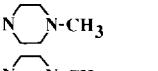
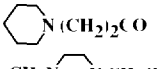
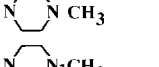
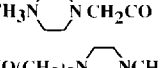
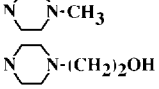
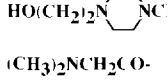
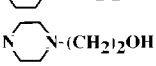
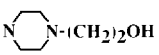
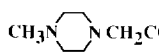
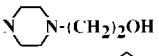
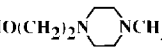
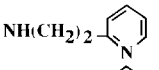
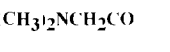
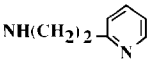
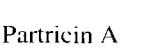
Compound	*R*	R_1	*Synthesis example*	*TLC* R_f	*TLC Method*	*HPLC RT (min)*	*HPLC Method*	*THC (μg/mL), rat RBC, 4 h at 36.5 °C*	*MHC (μg/mL), rat RBC, 4 h at 36.5 °C*	LD_{50} *(mg/kg) ip (iv) mice*
1	$NHCH_3$	H	1	0.45	A	32.1	A	2	0.07	1.9
2	NHC_2H_5	H	1	0.53	A	36.6	A	2	0.2	3.3
3	NH-*n*-C_3H_7	H	1	0.58	A	41.2	A	2	0.2	3.8
4	NH-*n*-C_4H_9	H	1	0.64	A	45.5	A	18	0.2	10
5	NH-*n*-C_5H_{11}	H	1	0.69	A	49.2	A	>18	0.7	35
6	$NHCH_2CH{=}CH_2$	H	1	0.58	A	46.2	A	2	0.07	3.8
7		H	2	0.53	A	36.0	B	6	0.7	6.4
8		H	2	0.58	A	48.5	B	6	0.7	9.7
9		H	2	0.54	A	30.0	B	6	0.7	4.7
10	$NH(CH_2)_2N(CH_3)_2$	H	2	0.54	A	30.4	A	18	0.7	10
11	$NH(CH_2)_3N(CH_3)_2$	H	2	0.51	A	30.6	A	6	0.2	5.1
12		H	2	0.61	A	26.0	B	18	0.7	13
13		H	2	0.66	A	29.0	B	18	0.7	13
14		H	2	0.61	A	25.1	B	>18	0.7	10
15		H	2	0.55	A	26.2	B	18	0.7	7.7
16		H	2	0.56	A	25.0	B	6	0.7	7.7
17		H	2	0.42	A	22.9	B	6	0.7	5.8
18		H	2	0.55	A	29.6	B	>18	6	70
19		H	2	0.64	A	38.3	B	6	0.7	5.8
20		H	2	0.48	A	20.6	B	18	0.7	12

Table I. *Continued.*

Compound	*R*	*R'*	*Synthesis example*	*TLC* R_f	*TLC Method*	*HPLC RT (min)*	*HPLC Method*	*THC (μg/mL), rat RBC, 4 h at 36.5 °C*	*MHC*	*LD_{50} (mg/kg) ip (iv) mice*
21		H	2	0.30	A	20.1	B	6	0.7	5.1
22	$NH(CH_2)_2N(CH_3)_2$	$(CH_3)_2NCH_2CO$	3	0.25	B	25.2	B	>18	18	158 (69.3)[a]
23	$NH(CH_2)_2N(CH_3)_2$		3	0.26	B	28.7	C	18	6	44
24	$NH(CH_2)_2N(CH_3)_2$		3	0.12	B	10.8	C	18	2	77
25	$NH(CH_2)_2N(CH_3)_2$		3	0.09	B	21.8	C	18	0.7	60
26		$(CH_3)_2NCH_2CO$	3	0.40	B	19.7	C	2	0.2	3.6
27			3	0.34	B	20.3	C	6	0.2	3.6
28			3	0.18	B	8.7	B	2	0.2	3.8
29			3	0.13	B	21.5	B	0.7	0.2	2.7
30		$(CH_3)_2NCH_2CO$-	3	0.27	B	21.3	B	0.7	0.2	3.0
31			3	0.26	B	26.2	B	2	0.2	2.4
32			3	0.11	B	21.2	B	2	0.2	2.9
33			3	0.07	B	20.5	C	0.7	0.2	1.5
34		$(CH_3)_2NCH_2CO$	3	0.39	B	32.4	B	>18	18	>300
35			3	0.34	B	26.6	B	>18	6	>300
36	Partricin A OH	H		0.16	A	10.4	B	0.2	0.07	0.6
37	Mepartricin A OCH_3	H						18	0.7	15 (3.90)[b]
38	Amphotericin B							2	0.7	50 (4.77)[c]

[a]Administered iv as the hydrosoluble diaspartate salt. [b]Administered iv as sodium laurylsulfate complex. [c]Administered iv as sodium deoxycholate complex, see reference [3] (LD_{50} 5 mg/kg iv mice) and reference [14] (LD_{50} 4 mg/kg iv mice; LD_{50} 88 mg/kg ip mice).

other compounds, with a few exceptions, were a little less effective, as were the additional reference substances, amphotericin B (MIC, 1.2 μg/mL) and partricin A (MIC, 2.4 μg/mL).

The activity against *T vaginalis*, the only protozoal strain tested, was very high with MIC values lower than (MIC 0.3 μg/mL) (**1–3, 6**) or equal to (MIC 0.6 μg/mL) (**4, 10, 11, 14, 15, 26–33**) that of mepartricin. However, all were less effective than the starting compound partricin A (MIC, 0.075 μg/mL). Amphotericin B was moderately effective against *T vaginalis*, with an MIC of 38 μg/mL.

The hemolytic activity is expressed as total hemolytic concentration (THC) or minimum hemolytic concentration (MHC), ie, the lowest concentration giving total hemolysis or the lowest concentration still

Table II. Antifungal and antiprotozoal activity of derivatives of partricin A.

Compound	*MIC (ng/mL)*						*IC_{50} (ng/mL)*			*MIC (μg/mL)*				
	C albicans 73 (liquid)		*C albicans 73 (solid)*		*C albicans 200 (solid)*		*C albicans 205 (solid)*		*S cerevisiae 167 (liquid)*	*Mucor sp 263 (solid)*		*A niger 374 (solid)*	*T mentagrophytes 176 (liquid)*	*T vaginalis 415 (semi-solid)*
	24 h	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*	*7 h*	*24 h*	*48 h*	*4 days*	*5 days*	*48 h*
1	3.75	7.5	15	15	15	15	15	30	0.9	0.6	0.6	0.15	0.6	0.3
2	7.5	7.5	30	30	30	30	30	30	1.2	0.3	0.3	0.075	1.2	0.3
3	7.5	15	15	15	30	60	30	60	1.2	0.3	0.3	0.075	2.4	0.3
4	7.5	15	30	60	60	120	60	60	1.3	0.6	0.6	0.075	>9.6	0.6
5	30	30	120	240	120	240	120	240	3.5	4.8	4.8	0.3	>9.6	4.8
6	7.5	15	15	30	30	30	15	30	1.0	0.3	0.6	0.075	1.2	0.3
7	15	30	30	60	60	60	30	120	1.9	0.3	0.3	0.3	1.2	1.2
8	30	30	30	60	60	60	30	60	2.0	0.3	0.3	0.3	1.2	1.2
9	15	30	30	60	30	60	15	60	2.0	0.3	0.3	0.15	1.2	1.2
10	3.75	7.5	15	15	15	15	7.5	30	0.5	0.6	0.6	0.3	0.3	0.6
11	7.5	7.5	7.5	15	15	15	7.5	15	0.6	0.3	0.3	0.15	0.3	0.6
12	3.75	7.5	30	30	30	60	30	30	0.9	0.6	0.6	0.075	0.3	1.2
13	7.5	15	30	30	30	60	30	30	0.8	0.6	0.6	0.075	0.3	2.4
14	3.75	15	30	30	30	30	15	30	0.8	0.3	0.3	0.075	0.6	0.6
15	3.75	15	30	30	30	30	15	30	0.9	0.3	0.3	0.075	0.6	0.6
16	3.75	7.5	15	60	30	60	15	30	1.1	0.6	0.6	0.15	0.3	1.2
17	3.75	3.75	15	30	30	60	15	15	0.6	0.6	0.6	0.075	0.3	1.2
18	7.5	30	30	60	60	120	30	60	2.2	9.6	>9.6	0.15	>4.8	>9.6
19	7.5	30	30	120	60	120	30	60	2.0	0.15	0.15	0.15	1.2	1.2
20	3.75	7.5	15	60	30	60	30	60	0.7	0.6	1.2	0.075	0.3	1.2
21	3.75	7.5	15	60	30	60	15	30	0.6	0.6	1.2	0.075	0.6	1.2
22	7.5	15	15	60	30	60	30	60	1.1	1.2	2.4	0.3	0.3	2.4
23	7.5	15	15	60	30	120	30	120	1.7	4.8	4.8	0.3	0.6	2.4
24	7.5	30	30	60	60	120	30	60	1.5	0.6	1.2	0.15	0.3	2.4
25	7.5	30	30	120	60	60	30	60	1.6	1.2	1.2	0.15	0.3	2.4
26	7.5	7.5	15	30	60	60	15	60	1.0	1.2	1.2	0.3	0.6	0.6
27	7.5	7.5	15	60	60	120	30	60	1.2	2.4	4.8	0.6	0.6	0.6
28	7.5	15	15	60	30	60	30	30	1.4	0.6	0.6	0.6	0.6	0.6
29	7.5	15	30	30	60	60	30	30	1.4	0.6	0.6	0.15	0.3	0.6
30	7.5	7.5	15	30	60	60	15	60	1.1	0.6	0.6	0.3	0.6	0.6
31	7.5	15	15	15	30	60	30	30	1.1	1.2	2.4	0.15	0.6	0.6
32	15	30	30	60	60	60	30	60	1.9	0.6	0.6	0.3	0.6	0.6
33	7.5	15	30	60	60	120	30	60	2.1	0.6	0.6	0.3	0.6	0.6
34	15	30	120	480	240	480	120	480	3.9	>9.6	>9.6	>9.6	>9.6	>9.6
35	60	120	480	>960	>960	>960	>960	>960	7.0	>9.6	>9.6	>9.6	>9.6	>9.6
36	30	60	60	120	60	120	60	120	2.1	1.2	2.4	2.4	2.4	0.075
37	7.5	15	15	30	30	30	15	30	1.0	0.6	0.6	0.15	0.6	0.6
38	240	240	480	960	480	960	480	960	35.5	0.15	0.15	1.2	1.2	38.4

showing a detectable hemolytic activity. A noticeable activity was detected for all members of the series (table I). Although they all were less hemolytic than partricin A (THC = 0.2 μg/mL; MHC = 0.07 μg/mL), most were similar to amphotericin B (THC = 2 μg/mL; MHC = 0.7 μg/mL) and mepartricin A (THC = 18 μg/mL; MHC = 0.7 μg/mL). Only a few were better: by omitting a few microbiologically ineffective compounds (**34, 35**), and compounds with little effect on filamentous fungi (**18**), only **22–24** were definitely less hemolytic than amphotericin B, with THC of 18 μg/mL or higher and MHC of 2–18 μg/mL (hemolytic concentrations about nine times and three to 25 times higher respectively).

The above results have been confirmed in an in vivo acute toxicity test in mice. With the exclusion of the less effective compounds (**34, 35, 5** and **18**), the best tolerated products are **22–24**, with LD_{50} values (ip, mice) similar to (**23, 24**) or higher than (**22**) that of amphotericin B **38** (LD_{50} = 158 and 50 mg/kg for **22** and **38** respectively).

To avoid bioavailability problems, ie, lack of absorption due to precipitation of the products in the peritoneum, the test was repeated administering the aqueous solutions intravenously. When **22** was dissolved as the diaspartate salt, it was about 14 times less toxic than amphotericin B **38** (Fungizone) (LD_{50} = 69.3 and 4.77 mg/kg, respectively, expressed as weight of active ingredients).

Conclusions

To conclude with a general survey, secondary and tertiary amides of partricin A (**1–9**), particularly when substituted with a lower alkyl group (**1–3**), are in vitro more effective antifungals than amphotericin B. However, they are no less toxic and are difficult to dissolve for injectable formulations, a problem common to other polyene antibiotics.

The basic amides **10–21** are even more potent antifungals and **10** and **11** are the most effective. Furthermore, they may give salts that are water soluble, but their toxicity remains similar to that of amphotericin B.

Basic diamides **22–35** are somewhat less potent than the other derivatives, but they maintain a higher antifungal activity than amphotericin B and can lead to hydrosoluble salts. Moreover, a few compounds, particularly **22**, are definitely less hemolytic and less toxic in comparison to amphotericin B.

Due to high potency, which may reduce the therapeutic dose, and to tolerability data, we have designated partricin A 2-dimethylaminoethyl amide (**10** code SPA-S-710) and *N*-dimethylaminoacetyl partricin A 2-dimethylaminoethyl amide (**22** code SPA-S-752) for further development. Compound **22** can be made hydrosoluble in the form of diaspartate or diascorbate salts (SPA-S-753 and SPA-S-843, respectively).

Experimental protocols

Partricin A was obtained from our own fermentation laboratory by growing a new mutant strain of *S aureofaciens* NRRL 3878.

Analytical methods

Thin layer chromatography (TLC) was carried out on silica-gel 60 F254 plates (Merck), eluting with 1,2-$C_2H_4Cl_2$/C_2H_5OH/40% aqueous $(CH_3)_2NH$ 64:30:9 *(Method A)* or with CH_2Cl_2/CH_3OH/conc NH_4OH/H_2O 85:15:1:1 *(Method B)*; UV detection at λ = 254 nm.

High pressure liquid chromatography (HPLC) was carried out by means of a Perkin Elmer series 3 instrument with gradient programming *(Method A)* or a Merck-Hitachi Model L 6200 chromatograph equipped with two gradient pumps (*Methods B* and *C*). Both instruments adopted a UV detector at 378 nm, cell 8 μ x 1 cm, with integrator and a Hibar Lichrocart 125 mm column (diameter 4 mm, packed with Superspher RP-18 4 μ) operating at room temperature, with a mobile phase made of a mixture of acetonitrile and 5 mM EDTA aqueous solution at a constant flow rate of 1 mL/min.

Method A adopted isocratic 35% acetonitrile for 15 min, gradient curve 2–65% for 45 min, linear gradient to 80% for 10 min followed by decrease to the starting conditions (35% acetonitrile) for 10 min and reequilibration of the column for 10 min

Method B used a linear gradient from 35 to 40% acetonitrile for 35 min, isocratic 40% acetonitrile for 20 min, a decrease to 35% within 1 min and reequilibration of the column for 10 min. *Method C* used isocratic 35% acetonitrile (10 min), a linear gradient to 45% (15 min), isocratic 45% acetonitrile (10 min) and then a decrease to the starting concentration of 35% in 1 min and reequilibration for 10 min.

UV/visible, IR and mass spectra were obtained with standard spectrometers, where appropriate. Elemental analysis (C, H, N) was performed with all derivatives and results were within ±0.4% of theoretical values.

Synthesis

Example 1. Partricin A methyl amide ***1***

To a solution of 6.6 g of partricin A in 55 mL dimethylacetamide, 0.89 g of methylamine dissolved in 10 mL dimethylacetamide was added at room temperature under stirring followed by 2.9 g triethylamine and 7.9 g diphenylphosphorazidate. The mixture was then stirred at room temperature for 4 h; the reaction progress was checked by thin layer chromatography. At the end of the reaction, the reaction mixture was treated with 500 mL of an ether/ethanol 9:1 mixture and the precipitate obtained was filtered off, washed with ether and dried at 40 °C under vacuum, yielding 5.8 g of product. The crude substance was then purified by means of column chromatography on silica gel, eluting with methylene chloride/methanol/diethylamine/water 86:10·4:1. After TLC assay, the suitable eluate fractions were evaporated to dryness under reduced pressure to give the required partricin A methyl amide (2.1 g) as a yellow-coloured crystalline solid. TLC, R_f 0.45 *(Method A)*; HPLC, RT 32.1 min *(Method A)*.

Example 2 Partricin A 2-dimethylaminoethyl amide ***10***
To a solution of 40 g of partricin A in 260 mL dimethylacetamide, 6.1 g of 2-dimethylaminoethylamine was added under stirring, followed by the dropwise addition of 19 1 g diphenyl phosphorazidate. The exothermic reaction was moderated by cooling to 15 °C and stirring at the same temperature for 2 h At the end of the reaction, a further 6.1 g 2-dimethylaminoethylamine and 19.1 g diphenylphosphorazidate were added, under cooling at 15 °C After stirring for 2 h, the reaction mixture was treated with 2.5 L of water and the resulting precipitate was collected by filtration, thoroughly washed with 200 mL ethanol and 800 mL water, and dried at 50 °C under vacuum, yielding 42 g of crude product.

Small amounts of unreacted partricin A, if present at the TLC assay, were eliminated by dissolving the product in 1 L of a methylene chloride/methanol 4:1 mixture, treating the solution with 40 g of Duolite LES resin under slow stirring overnight, and filtering off the resin.

The solution obtained was then subjected to a column chromatography on silica gel 60, 70-230 mesh (400 g), eluting first with methylene chloride/methanol/water 72:24.4 and then with methylene chloride/methanol/triethylamine/water 72.24·4:4. After TLC assay of the various fractions, 18 g of the expected partricin A 2-dimethylaminoethylamide was obtained by concentration under reduced pressure as a yellow crystalline solid. TLC, R_f 0.54 *(Method A)*; HPLC, RT 30.4 min *(Method A)*.

Example 3. N-Dimethylaminoacetyl partricin A 2-dimethylaminoethyl amide ***22***
Partricin A 2-dimethylaminoethyl amide (12.0 g) was dissolved in 110 mL dimethylacetamide. To this solution, 5.1 g dimethylaminoacetic acid, 5.0 g triethylamine and 13.8 g diphenyl phosphorazidate were successively added under stirring and at room temperature. The solution was kept for 6 h under stirring at room temperature, the progress of reaction being checked by TLC on silica-gel 60, F 254 plates (Merck). The reaction mixture was then treated with 1 L of ether/ethanol mixture 9:1 The precipitate was filtered off, washed with ether and dried under vacuum at 40 °C. The resulting raw product (about 12 g) was purified preferably by medium-pressure chromatography (MPLC) using silica gel in a weight ratio of 12:1 to the raw product and a methylene chloride/methanol/conc ammonium hydroxide/water 85:15:1:1 mixture as eluting system.

The MPLC fractions, pure to TLC, were pooled and evaporated to dryness under vacuum. The pure product (7.6 g) was obtained in the form of a deep yellow crystalline powder TLC, R_f 0.25 *(Method B)*; HPLC, RT 25.2 min *(Method B)*.

Biological evaluation

Antifungal agents and microorganisms
All derivatives were kept in sealed vials at –25 °C and for microbiological testing were dissolved in dimethylsulfoxide at 2000 μg/mL (DMSO, Merck) and serially diluted under stirring to the required concentrations. Mother solutions in DMSO were preserved at –25 °C in the dark for not more than 5 days.

The microbiological activity was assayed against three strains of *C albicans* (LRM 73, LRM 200 and ATCC 14053 (which we called LRM 205)), one strain of *S cerevisiae* (ATCC 9763 (called LRM 167)), three filamentous fungi (*Mucor* sp LRM 263, *A niger* ATCC 16404 (called LRM 374), *T mentagrophytes* ATCC 9129 (called LRM 176)) and one protozoa (*T vaginalis* LRM 415).

MIC against C albicans in liquid medium
The culture medium (Fluid Sabouraud Medium, DIFCO) was inoculated with about 10^4 cells/mL of *C albicans* (broth culture of 16 h at 35 °C). In sterile 16 x 160 mm glass test tubes, 0.5 mL from 1:2 serial dilutions of the products were treated with 4.5 mL inoculated culture medium. The cultures were incubated at 35 °C for 24 and 48 h. The readings were made with the naked eye and the microorganism growth was evaluated according to the degree of culture turbidity and in comparison with the turbidity of the control test tube containing no antifungal agent. The MIC is represented by the lowest concentration not giving any microbial growth.

MIC against C albicans in solid medium
In sterile, plastic, 100 mm diameter Petri plates, 1.5 mL portions of 1.2 serial dilutions of the products were treated with 13.5 mL of Fluid Sabouraud Medium (DIFCO) supplemented with DIFCO Bacto agar (1.5%). From yeast broth cultures of 16 h at 35 °C with 20% transmittance, suitable dilutions were performed to obtain a suspension containing 10^5 cells/mL. The resulting suspension (10 μL) was then seeded on the medium surface (inoculum of about 10^3 cells). The plates were incubated at 35 °C for 24–48 h wrapped in parchment paper to decrease the evaporation of water from the culture medium. The readings were made with the naked eye and the microorganism growth in the sown zone was evaluated against growth on the control plate containing no antifungal agent.

IC_{50} against S cerevisiae in liquid medium
The tests were carried out in PYG medium (Oxoid peptone P, Difco yeast extract, Merck glucose) adjusted to pH 7 with 40% NaOH. One millilitre from 1:2 serial dilutions in culture medium of the products in sterile 18 x 180 mm glass test tubes was treated with 9 mL of the same medium inoculated with an *S cerevisiae* suspension (agar culture of 16 h at 35 °C), in triplicate for each dose level. The inoculum was of 4 x 10^5 cells/mL. The cultures were incubated in a water bath at 36 5 °C for about 7 h, and growth was stopped using 0.5 mL of 15% formalin. The turbidity of the cultures was measured with a spectrophotometer at 580 nm. By converting optical densities to percentage growth as compared to the control and plotting these percentages against the dose logarithms, it was possible to plot a line comprising 50% of the growth; the inhibitory concentration at this value was described as IC_{50}.

MIC against Mucor sp and A niger in solid medium
In sterile, plastic, 55 mm diameter Petri plates, 0.5 mL portions from 1:2 serial dilutions of the products were treated with 4.5 mL of culture medium (Sabouraud Dextrose Agar, DIFCO). The mycete spore suspensions in saline solution, stored at 4 °C, were diluted so as to contain 5 x 10^5 spores/mL in the case of *Mucor* and 2.5 x 10^5 spores/mL in the case of *Aspergillus*. 10 μL of the suspension was then seeded After allowing the drop to dry for 10 min under a laminar flow, the plates were incubated at 30 °C in a humid environment to limit evaporation of water from the culture medium The readings were performed with the naked eye, after 24 and 48 h in the case of *Mucor* and after 4 days in the case of *Aspergillus*, growth was evaluated by comparison with the control plate containing no antifungal agent.

MIC against T mentagrophytes in liquid medium
The culture medium (Fluid Sabouraud Medium, DIFCO) was inoculated with approximately 3 x 10^4 cells/mL, starting from a conidium suspension in a suitably diluted saline solution, stored at 4 °C. In sterile 16 x 160 mm glass test tubes 0 5 mL

from 1.2 serial dilutions of the various products were added with 4.5 mL of culture medium inoculated with 3 x 10^4 conidia/ mL. The cultures were incubated at 30 °C for 5 days; the readings were performed with the naked eye; the microorganism growth was evaluated against the control test tube containing no antifungal agent.

MIC against T vaginalis in semisolid medium
Serial dilutions (1:2) of the products (0.8 mL) were mixed with CPLM Biolife medium (7.2 mL) preserved at 50 °C, in sterile 18 x 180 mm glass test tubes. After cooling each tube was seeded with 0.1 mL of a 48–60 h culture of *T vaginalis*, in the central portion of the liquid column. The cultures were incubated at 35 °C, gently shaken after 24 h, incubated for additional 24 h and then read. The protozoan growth was evaluated from the culture turbidity compared to the turbidity of the control tube containing no antifungal agent.

THC and MHC against rat red blood cells
Blood was collected by cannulating the abdominal aorta from fasting male rats under Et_2O anesthesia. The blood was collected in an equal volume of Alsever anticoagulant liquid (composition (mM): glucose 114, sodium citrate 31, NaCl 72, citric acid 2.8, all from Merck), sterilized by filtration through a 0.22 μ Millipore Sterifil-D unit fitted with a Durapore membrane.

The red blood cells (RBC) suspension stored at 4 °C for not more than 1 week, was diluted 1:2 with a 2 g/L solution of FV bovine serum albumin (BSA, Sigma A-7030) in a pH 7 Ringer-like solution (composition (mM): NaCl 154, KCl 2, $CaCl_2 \cdot 2H_2O$ 1, all from Merck). The resulting suspension was centrifuged at 2500 rpm for 6 min, and subsequently the sediment was gently resuspended in the same BSA Ringer. Washing was repeated three times, each time resuspending the cells in an equal volume of solution. Finally, the suspensions were centrifuged for 15 min at 2500 rpm in graduated test tubes. The quantity of sediment was measured and the cells were then suspended at 2.8% in the same diluent.

The products were dissolved in DMSO (Merck) at 1000–4000 μg/mL and suitably diluted 1:2 by quick addition of distilled water (the test tube being immediately shaken to avoid precipitation of the polyene) and then of the above medium.

Each glass test tube (16 x 100 mm) containing 0.1 mL of the polyene dilutions and 2.9 mL of BSA Ringer was added to 1 mL of 2.8% RBC suspension so as to obtain a final RBC concentration of 0.7%. The tubes were sealed with parafilm and incubated in a water bath at 37 °C for 4 h in the dark, each tube being gently shaken every 20–30 min in order to avoid excess sedimentation of the RBC, and soon after were briefly centrifuged (2500 rpm for less than 1 min).

The hemolysis degree was evaluated by comparison of the solutions by the naked eye and under artificial light with the colour of hemoglobin solutions at different concentrations, obtained by lysis with distilled water and 10% ammonia of serially diluted RBC suspensions.

Acute toxicity in mice by intraperitoneal administration
Male CD1 mice (Charles River), about 4 weeks old and weighing 14–18 g when received, were housed at controlled conditions of 21 ± 1 °C, 60 ± 5% relative humidity, artificial lighting with a circadian cycle of 12 h of light and were fed a standard diet (Piccioni Laboratories) for a week, before starting the tests.

Groups of mice were treated by intraperitoneal route with different amounts of each test polyene dissolved in 0.2 mL dimethylsulfoxide, diluted with at least 10 mL water, and administered at the constant volume of 10 mL/kg.

In a few cases the products were administered intravenously in aqueous solution. **22** was dissolved as the diaspartate salt and the reference compounds mepartricin A **37** as sodium laurylsulfate complex [12] and amphotericin B **38** as sodium deoxycholate complex (Fungizone).

The animals were then controlled for 15 days, by recording any mortality and behavioural change.

The toxicity of the products was calculated by the Probit method [13], as approximate dose lethal to 50% of the animals (LD_{50}, mg/kg body weight).

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