What's new in Polyene Macrolide Antifungals?

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Fungal Infections

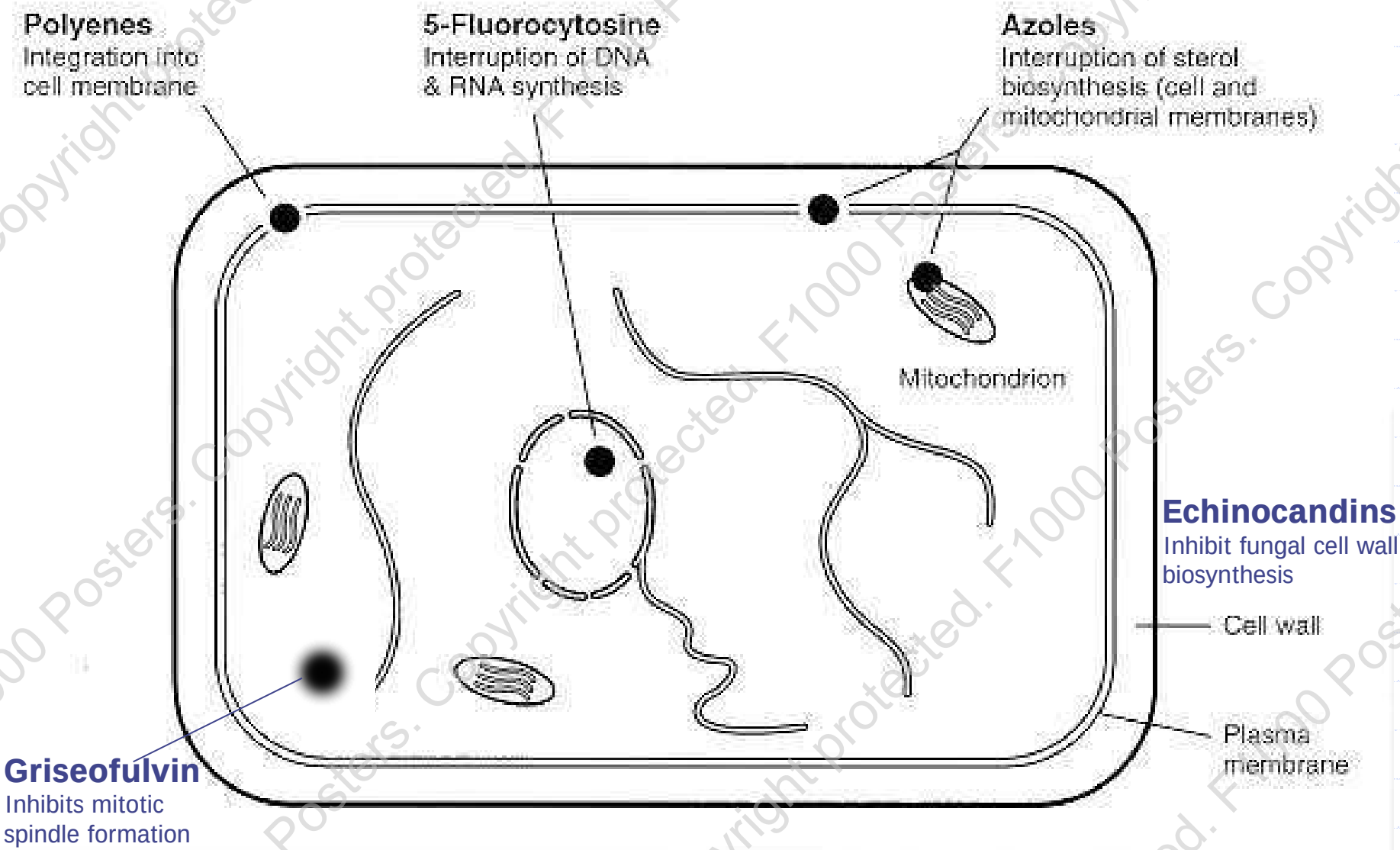
Yeasts

- *Candida albicans*
- Non-albicans *Candida* spp.
- *Cryptococcus neoformans*, *C. gatti*
- *Trichosporon* spp., etc.

Molds

- Hyaline molds – *Aspergillus* spp., *Fusarium* spp.
- Dematiaceous mold – *Scedosporium* spp., etc
- Dimorphic Fungi – *Histoplasma capsulatum*, etc.
- Dermatophytes – *Trichophyton* spp., etc.
- Zygomycetes – *Rhizopus oryzae*, etc.

Antifungal Agents- Sites of action



Polyene Macrolide Antibiotics

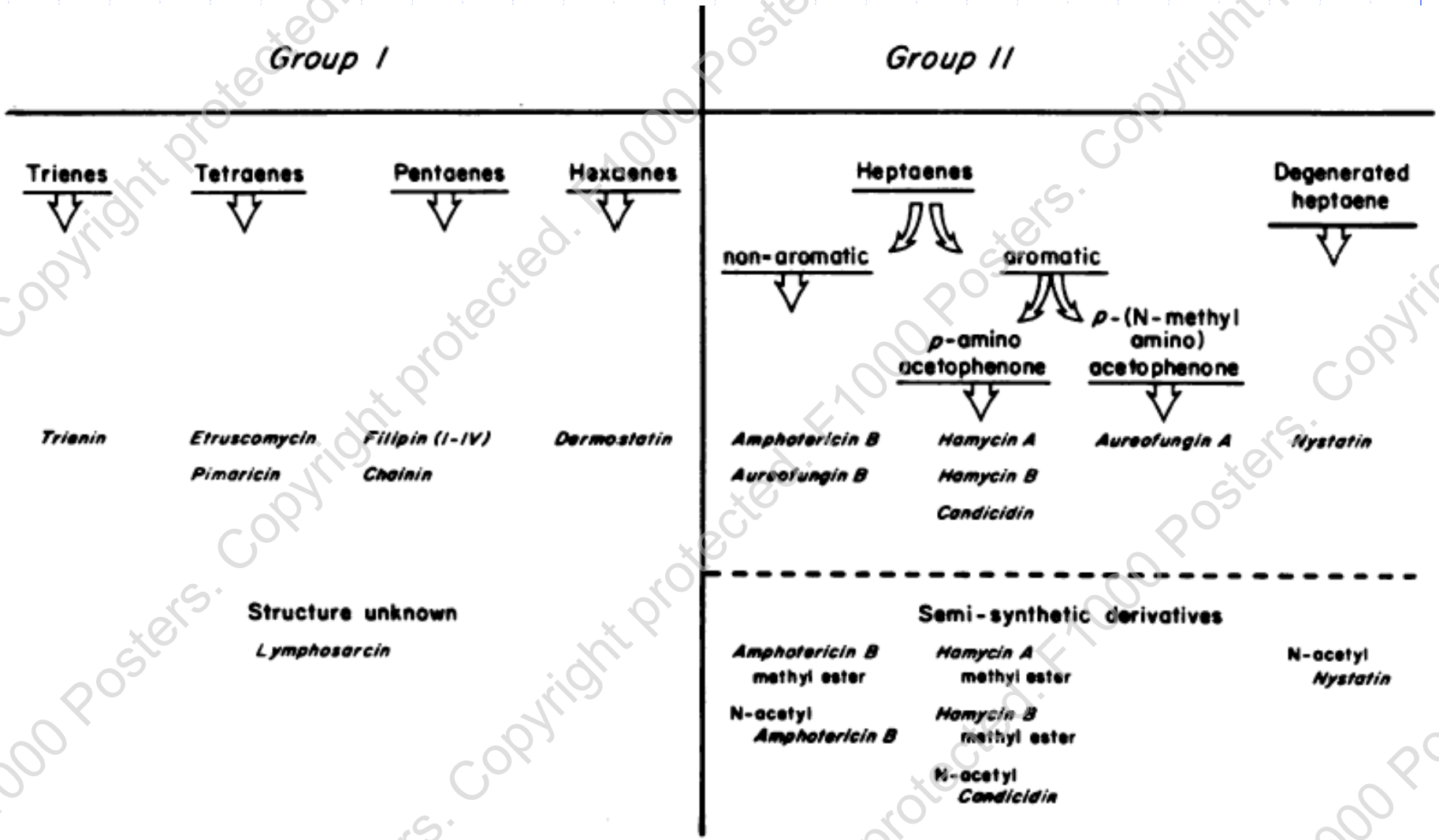


FIG. 6. *Classification of polyene macrolide antibiotics according to chemical structure and biological effects.*

Kotler-Brajtburg J, et al. 1979. Antimicrobial Agents Chemothera. 15 (5): 716-722

Polyene Antifungal Agents

- Interact with ergosterol in the fungal cell membrane and form pores

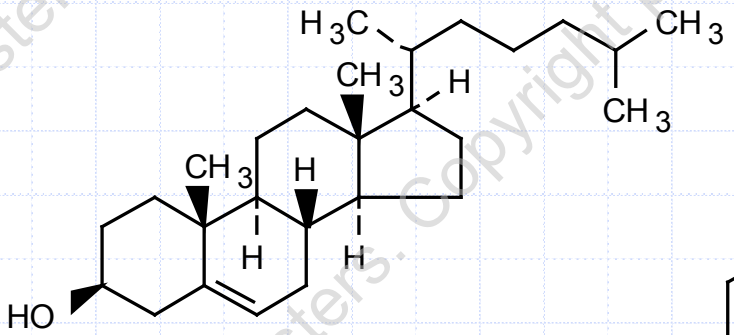
- Polyenes related macrolide antibiotics with the large lactone ring but have lipophilic (a chromophore of 4-7 conjugated double bonds) and hyrophilic side (several alcohols, acids and usually a sugar).

- Conjugated double bonds correlates with antifungal activity in vitro and inversely with the degree of toxicity to mammalian cells.

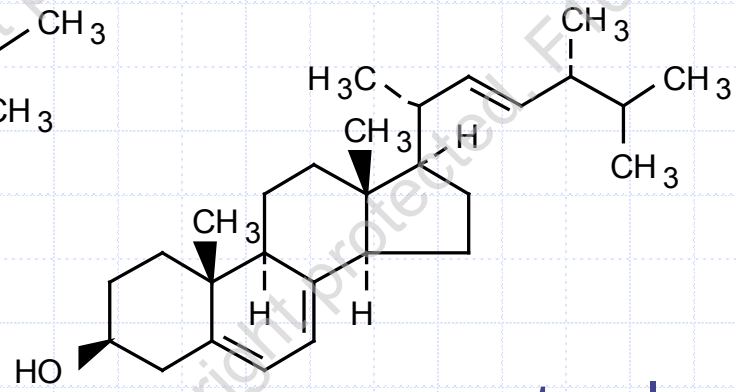
  - Amphotericin B
  - Nystatin
  - (Natamycin) Pimaricin

Mechanism of Action of Polyenes

- Polyenes bind to fungal membrane sterols
- Polyenes have a high affinity for ergosterol
- They insert into the membrane and disrupt membrane function Then membranes become leaky
- Ergosterol is not present in mammalian membranes
- Recent thinking is that the polyenes form small transmembrane pores that allow K to leak through
- The polyenes are fungicidal as they kill the fungi
- Polyenes can bind to cholesterol (present in mammalian cell membrane)

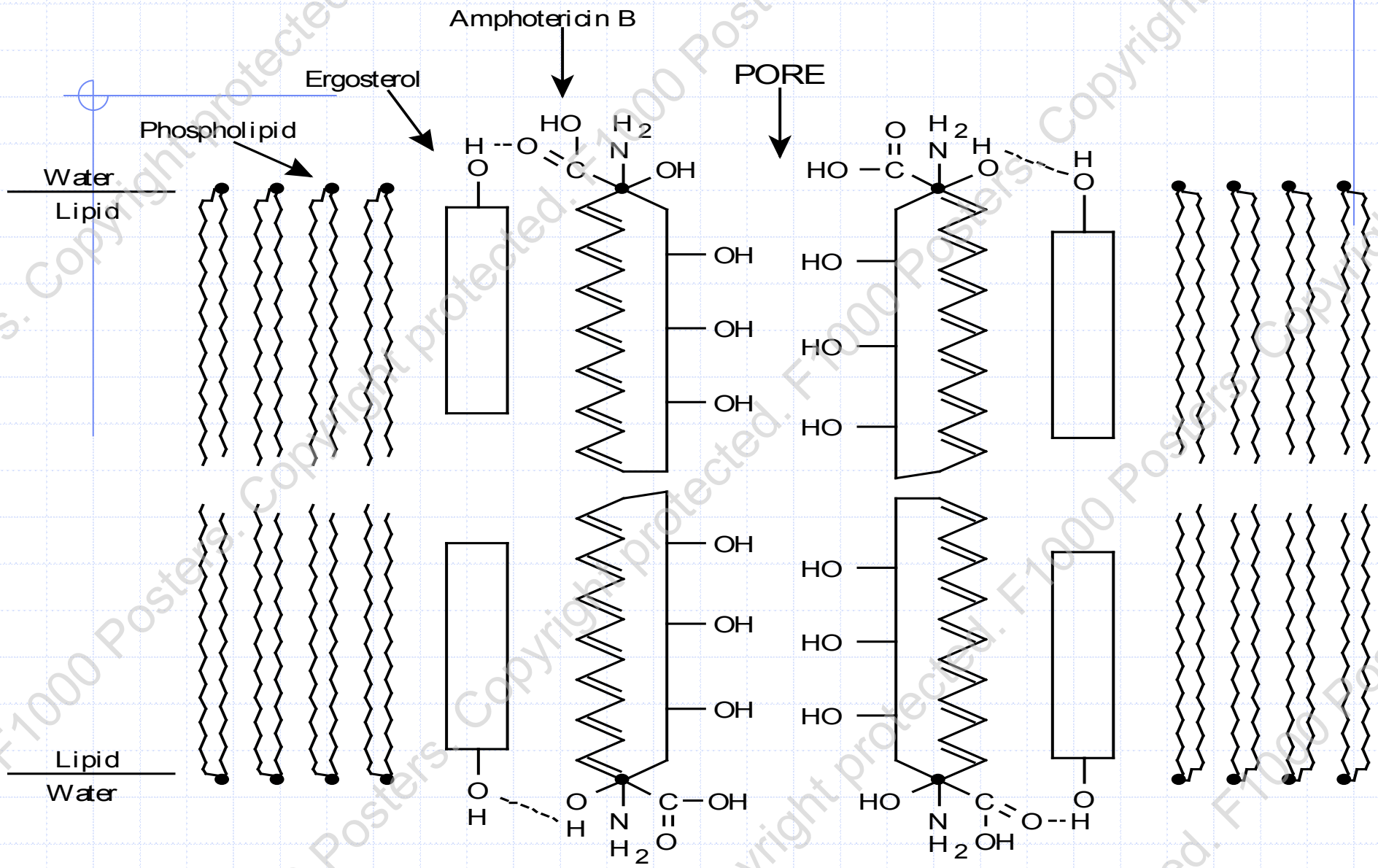


cholesterol

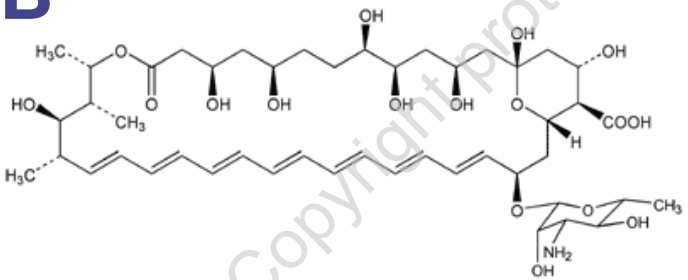


ergosterol

Mechanism of Action of Polyenes



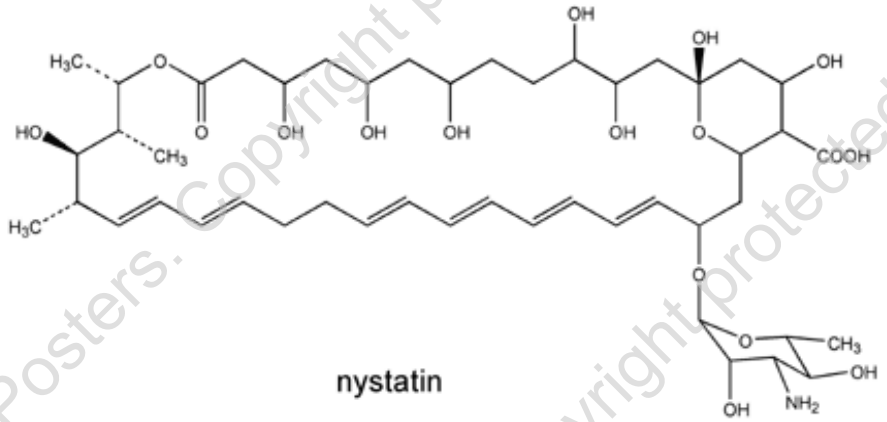
Amphotericin B



- Polyene antibiotic
- Produced by *Streptomyces nodosus*
- Discovered in 1956
- For 30 years the main available drug to control serious fungal infections.
- It is indicated for treatment of severe, potentially life threatening fungal infections.
- It must be given IV and is toxic (due to nonselective action on cholesterol in mammalian cell membranes)
- Serious fungal infections involve long therapy
- Resistance is due to lower production of membrane sterols or altered sterols, but is relatively rare at present
- Target modification and reduced access to target are other mechanisms of resistance.

Nystatin

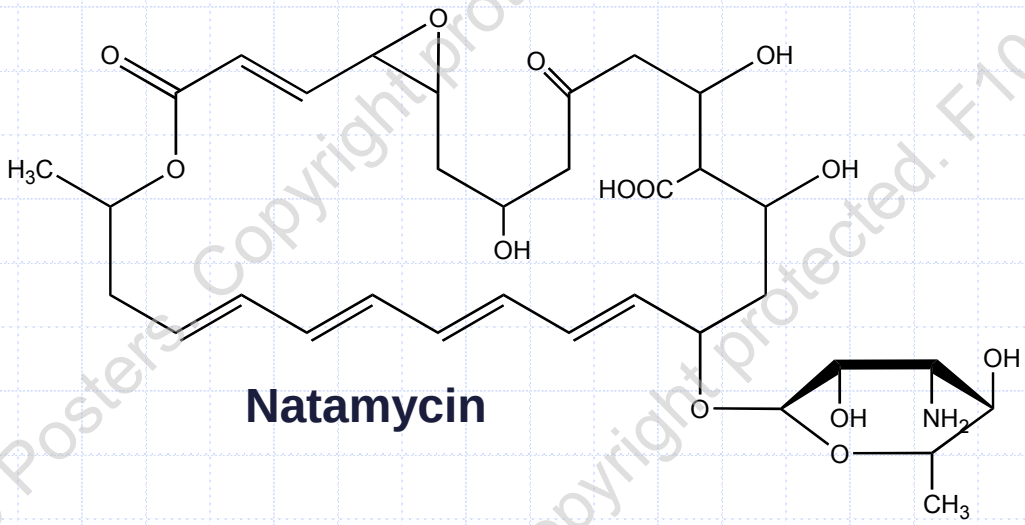
- Isolated from streptomyces noursei in 1951
- Conjugated tetraene polyene
- First clinically useful polyene antifungal antibiotic
- Available in oral tablets, powder for suspension, vaginal tablets, pastilles
- This polyene is used for local therapy only (not absorbed)
- For gut Candidiasis, and in a "swish and swallow" routine for oral Candidiasis
- No significant adverse effects with these uses



nystatin

Natamycin

- Polyene antibiotic obtained from cultures of *Streptomyces natalensis*
- Structures consists of 26-membered lactone instead of the 38 for Nystatin and Amphotericin B
- The 26-membered polyenes cause both K leakage and cell lysis at same concentration.
- Natamycin supplied as a 5% ophthalmic suspension intended for the treatment of fungal conjunctivitis, blepharitis and keratitis



Natamycin

Lipid formulations of polyenes

Lipid-based formulations of the polyene macrolide amphotericin B

Improving efficacy in invasive fungal infections

- Amphotericin B
  - –The most widely used antifungal for systemic infections
  - –High level of toxicity
- Nystatin
  - –Significant nephrotoxicity
  - –Has not been developed to treat systemic fungal infections

Lipid formulations of polyenes

- Improve the therapeutic index for polyene macrolides
- AmB lipid complex
- AmB colloidal dispersion
- Liposomal amphotericin B
  - invasive fungal infections in patients refractory or intolerant to standard AmB
- Liposomal nystatin
  - phase III clinical trials

Lipid formulations of polyenes

- Advantages
  - Broad spectrum
    - *Candida* spp.
    - *Cryptococcus neoformans*
    - *Aspergillus* spp.
  - Less toxicity in vivo
- Disadvantages
  - Increased cost
  - Not active for dermatophyte infections

		Study design	Treatment groups	Patient number	Duration of treatment	Response at end of therapy
Candidiasis	IC, second-line treatment	MC	LAmB 0.5–5 mg/kg/day	25	18 days	76% cured
	IC in neonates and preterms, first-line treatment	SC	LAmB 1–5 mg/kg/day	44	22 days	73%
	IC in very low birth weight infants, first- or second-line treatment	MC	LAmB 2.5–7 mg/kg/day	24	2 weeks	83%
	Candidaemia and IC, first-line treatment	MC, R, DB	LAmB 3 mg/kg/day	202	2 weeks	89.6%
			Micafungin 100 mg/day	190		89.5%
	Candidaemia in premature infants, first-line treatment	SC	LAmB 5 mg/kg/day	6	2 weeks	83.3%
			ABCD 3 mg/kg/day	16		57.1%
			AmBD 1 mg/kg/day	34		67.6%
Aspergillosis	Proven IA, second-line treatment	MC	LAmB 0.5–5 mg/kg/day	28	29 days	32% cured
	IA and neutropenia, first-line treatment	MC, R	LAmB 1 mg/kg/day	41	2 weeks	64%
			LAmB 4 mg/kg/day	46		48%
	IFI (53 IA), first-line treatment	MC, R	LAmB 5 mg/kg/day	25	2 weeks	52%
			AmBD 1 mg/kg/day	28		29%
	IFI (95% IA) 'Ambiload' trial	MC, R, DB	LAmB 3 mg/kg/day	107	2 weeks	50%
			LAmB 10 mg/kg/day	97		46%
	IA, first-line treatment	MC, R	LAmB 10 mg/kg/day	15	17 days	27%
			LAmB 3 mg/kg/day + caspofungin	15		67%
Histoplasmosis	Disseminated in patients with AIDS	MC, R, DB	LAmB 3 mg/kg/day	55	2 weeks	88%
			AmBD 0.7 mg/kg/day	26		64%
Cryptococcosis	Cryptococcal meningitis and AIDS	MC, R	LAmB 4 mg/kg/day	15	3 weeks	86%
			AmBD 0.7 mg/kg/day	13		80%
	Cryptococcal meningitis and AIDS	MC, R, DB	LAmB 3 mg/kg/day	267	2 weeks	64%
			LAmB 6 mg/kg/day			54%
			AmBD 0.7 mg/kg/day			54%
Febrile neutropenia	Febrile neutropenia	MC, R, DB	LAmB 3 mg/kg/day	343	Neutropenia	50%
			AmBD 0.6 mg/kg/day	344		49%
	Febrile neutropenia	MC, R, DB	LAmB 3 mg/kg/day	422	3 days after neutropenia resolution	31%
			VRZ 6 mg/kg twice-daily D1, 3 mg/kg twice-daily	415		26%
	Febrile neutropenia	MC, R, DB	LAmB 3 mg/kg/day	539	7 days after neutropenia resolution	34%
			Caspofungin 70 mg/day, 50 mg/day	556		34%
	Febrile neutropenia	MC, R, DB	LAmB 3 mg/kg/day	85	3 days after neutropenia resolution	42%
			LAmB 5 mg/kg/day	81		40%
			ABLC 5 mg/kg/day	78		33%
IFI prophylaxis	AL	MC	LAmB 10 mg/kg/week	15	4 weeks	AE: 2.9/patients
	ASCT			8	8 weeks	AE: 7.2/patients
	ACST acute GVHD	SC	LAmB 7.5 mg/kg/week	21	8 weeks	AE: 33%
	ACST children	MC	LAmB 3 mg/kg/day	51	100 days	Dose decrease: 30% Treatment interruption: 11%

F. Lanternier & O. Lortholary. Clin Microbiol Infect 2008; 14 (Suppl. 4): 71–83.

Candicidin

- Aromatic polyene – heptaene antibiotic
- Produced by Streptomyces griseus IMRU 3570
- First described by Lechevalier et al in 1953; named as C135
- Strong activity against Candida species
- Was used to treat vaginal candidiasis & prostatic hyperplasia
- Not used today due to less solubility and high toxicity

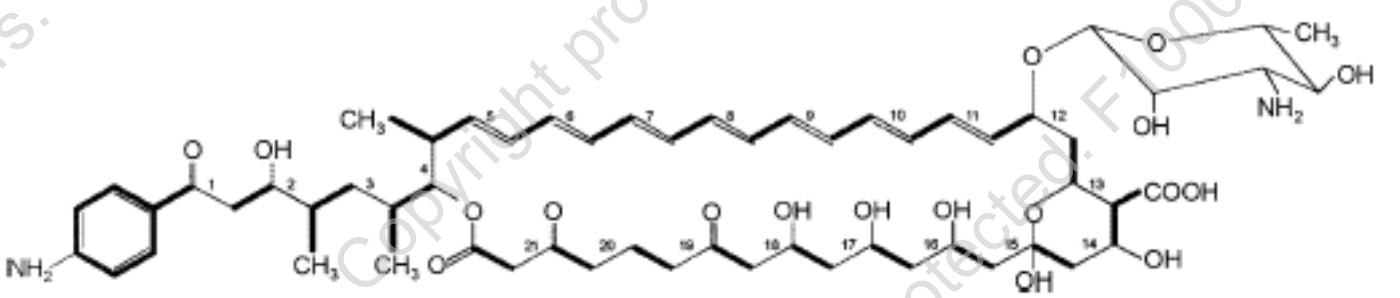


Fig. 1. Structure of the candicidin-D molecule (Zielinski *et al.*, 1979).

Gil JA & Campelo-Diez AB. 2003. Appl Microbiol Biotechnol. 60: 633-642.

Filipin

- A polyene macrolide compound
- Isolated by scientist (Upjohn company) in 1955 from *Streptomyces filipinensis*,
- In a soil sample collected in the Philippine Islands, hence the name Filipin.
- Has potent antifungal activity
- Toxic, not used in humans, however used in food spoilage and crop infection

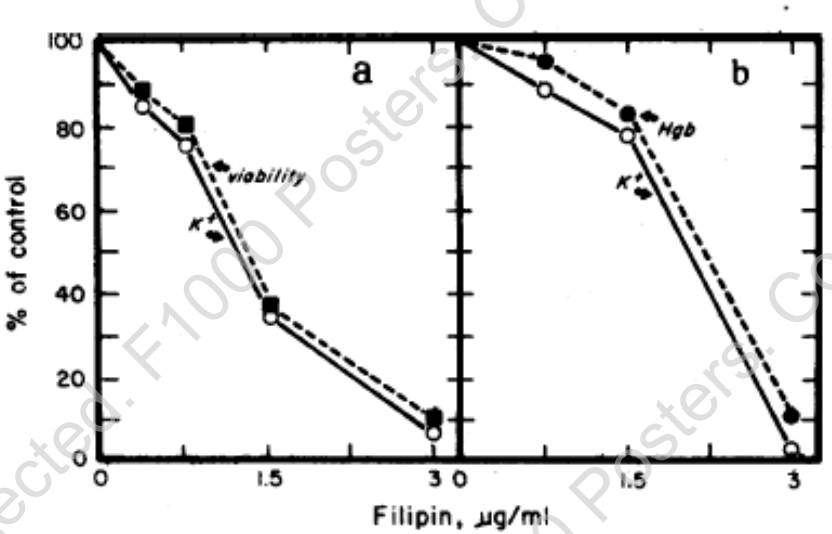


FIG. 3. ***Effect of filipin on (a) yeast and (b) RBC. Symbols: percentage of: ○, remaining K^+; ■, colony-forming units; ●, remaining hemoglobin.***

Mepartricin (methyl ester partricin)

- Partricin, SPA-S-132 produced by *Streptomyces aureofaciens* (NRRL 3878)
- Macrolides similar to amphotericin
- Partricin is very active against fungi and protozoa: minimum
- inhibitory concentrations (MIC) on *Candida albicans*
- were about 0.2 μg/ml.
- It is tolerated by oral route, but is very toxic in mice (IV) and shows a high hemolytic activity
- To improve the biological properties of partricin, methyl ester was prepared
- Currently used in the treatment of chronic prostatitis, vaginitis

H. Lechevalier, E. Borowski, J. O. Lampen and C. P. Schaffner. 1961. Antibiotics Chemother. 17, 640

Trichomycin

- Heptaene macrolide, a potent and clinically useful antifungal drug
- Isolated from *Streptomyces hachijoensis*
- Two components, trichomycins A and B

Table 1. MIC and spectra of trichomycin A and amphotericin B against yeasts, fungi and trichomonas.

Organism	MIC (μg/ml)	
	Trichomycin A	Amphotericin B
Candida albicans (eight strains)	Mean 0.034	Mean 0.25
C. tropicalis FP583	0.1	0.78
C. pseudotropicalis FP584	<0.025	0.78
C. krusei FP585	0.1	0.78
C. parakrusei FP586	0.2	0.78
C. guilliermondii FP587	0.1	0.78
C. stellatoidea FP588	<0.025	<0.025
Aspergillus niger FP1398	0.1	1.56
A. flavus FP1022	6.25	1.56
A. fumigatus FP1305	6.25	1.56
Trichophyton rubrum FP596	12.5	3.13
T. mentagrophytes FP594	6.25	3.13
Cryptococcus neoformans FP1551	<0.025	<0.025
Trichomonas vaginalis 4FM[a]	0.2	>100
T. vaginalis ATCC 30001[a]	0.2	>100
T. vaginalis PCL110108[a]	0.2	>100

[a] V-Bouillon.

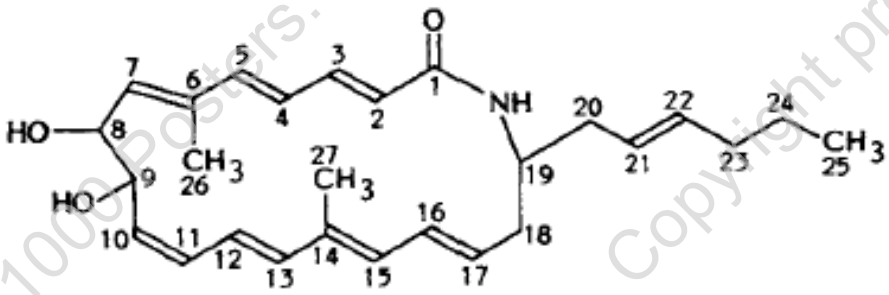
Komori T, Morimoto Y. 1990. The J Antibiotics. XLIII;7: 904-906

Newer Polyenes

- BE-14106
- S44HP
- SJA-95
- SPK-843
- TPU-0043
- YS-822A

BE-14106

- Macrocyclic lactam antibiotic
- While screening for antitumor substances from soil samples collected in Shiroyama-cho, Mie Prefecture, Japan
- From *Streptomyces spheroides*.
- Inhibited the growth of murine leukemia cell line
- Also exhibited antimicrobial activity
- Toxic to use in humans



Test organism	MIC (μg/ml)
Pseudomonas aeruginosa IFO 3445	100
Flavobacterium meningosepticum IFO 12535	100
Wicherhamia fluorescens IFO 1116	12.5
Saccharomyces cerevisiae IFO 0283	6.25
Schizosaccharomyces pombe IAM 4863	3.13
Candida albicans IFO 1270	25
Endomyces ovetensis IFO 1201	100

Kojiri K, Nakajimat S, Suzuki H, Kondo H, Suda H. 1992. J Antibiotics. 45 (6): 868 – 874.

S44HP

- Genetically engineered by alteration in the polyol region and exocyclic carboxy group
- Biosynthesis genes in *Streptomyces noursei*
- Tested in vitro against yeasts (*Candida albicans* etc.) and molds (*Aspergillus niger*)
- Less toxic in animal model as compared to amphotericin B
- Activity was as comparable to amphotericin B in animal model

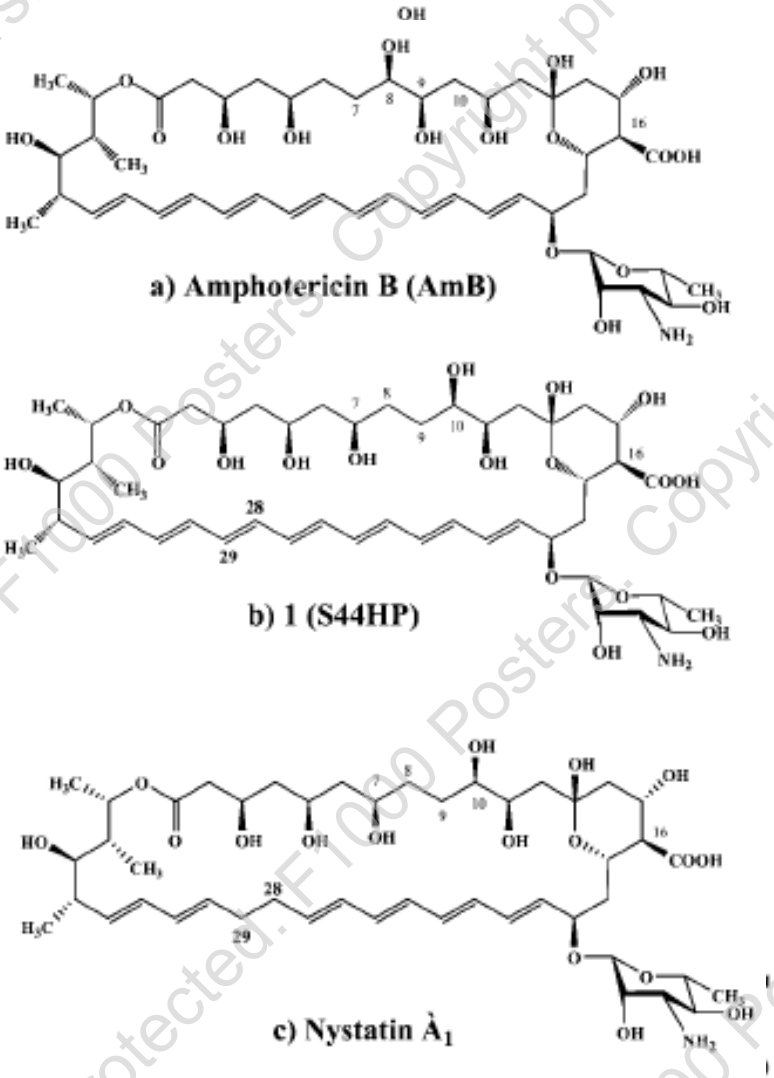


Figure 1. Chemical structures of amphotericin B (AmB) (a), 1 (S44HP) (b), and nystatin A_1 (c).

Preobrazhenskaya MN et al. 2009. J Med. Chem. 52: 189-196.

SJA-95

- Antifungal antibiotics from new strain of *Streptomyces* sp. S-24
- Heptaene polyene macrolide
- Antifungal activity in vitro against yeasts, filamentous fungi and clinical isolates
- Increased toxicity and lack of bioavailability at the infected site
- Liposomal SJA-95 was synthesized; less toxicity, warrants further investigation

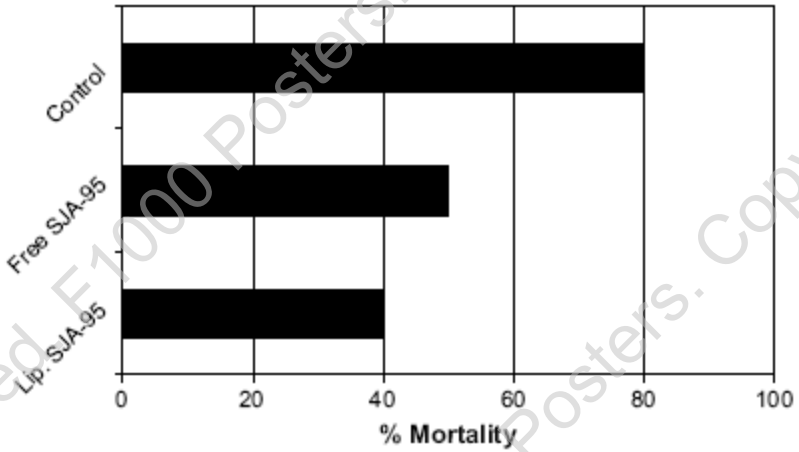


Fig. 1. Effect of SJA-95 treatment on cryptococcosis induced mortality in mice.

Desai SK and Nail SR. 2009. Biomedicine & Pharmacotherapy. 63: 287-292.

SPK-843

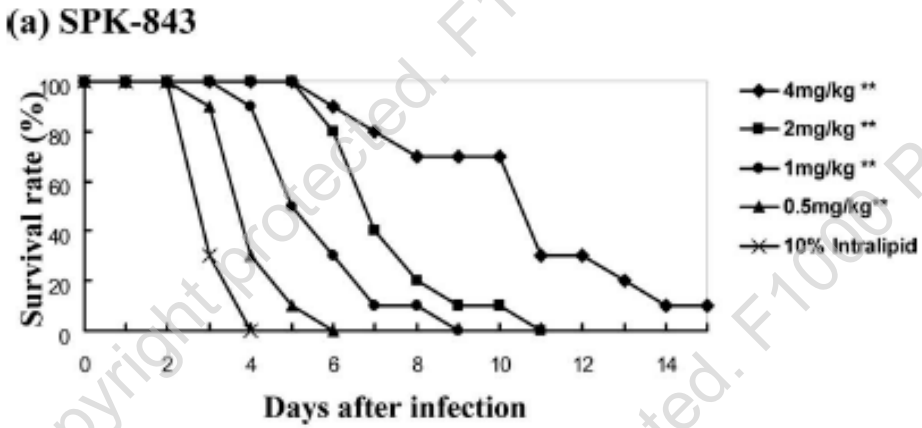
New polyene antifungal

Water soluble diascorbate salt from SPA-S-752

Amide derivative of partricin A, produced by mutant strain of *Streptomyces aureofaciens*

In vitro inhibitory activity comparable to amphotericin B against *Candida* sp., *Cryptococcus* neoformans, and *Aspergillus* sp.

In murine pulmonary aspergillosis, better survival prolongation as compared to amphotericin B and liposomal amphotericin B



Kakeya H et al. 2008.
Antimicrobial Agents Chemother.
52 (5): 1868 - 1870

SPA-S-843

- Series of partricin A amides were synthesized by active ester method - several amines on the carboxy group and then with some acids on the mycosamine group of partricin A

- This synthesized a new antifungal drug is *N*-dimethylaminoacetyl-partricin A (2-dimethylaminoethylamide diascorbate)

- New water-soluble partricin A derivative

- Developed by Societa` Prodotti Antibiotici (Milan, Italy)

- High antifungal potential with low toxicity

SPA-S-843 (Cont'd)

TABLE 1. In vitro activities of SPA-S-843 and amphotericin B against yeast strains

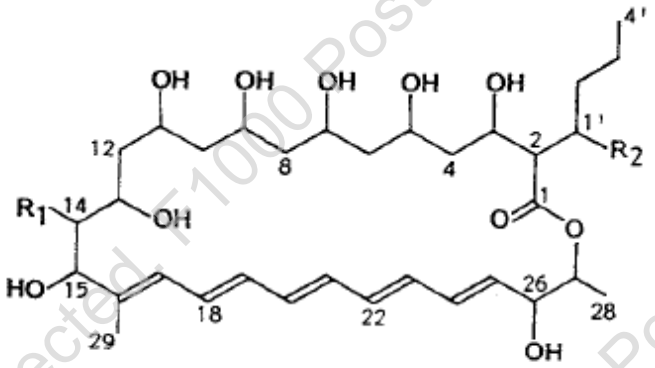
Organism (no. of strains)	Drug[a]	MIC (μg/ml)				MFC_{99} (μg/ml)	
		Range	50%	90%	GM	Range	GM
C. albicans (48)	SPA-S-843	0.009–0.15	0.075	0.15	0.054	0.009–2.4	0.094
	AmB	0.075–0.3	0.15	0.15	0.124	0.075–0.3	0.181
C. glabrata (11)	SPA-S-843	0.019–0.075	0.038	0.075	0.042	0.15–2.4	0.386
	AmB	0.15–0.6	0.3	0.3	0.264	0.3–0.6	0.529
C. krusei (10)	SPA-S-843	0.019–0.3	0.075	0.3	0.08	0.019–0.6	0.131
	AmB	0.15–0.3	0.3	0.3	0.227	0.15–1.2	0.345
C. parapsilosis (4)	SPA-S-843	0.038–0.075				0.3–4.8	
	AmB	0.075–0.3				0.3–0.6	
C. tropicalis (12)	SPA-S-843	0.019–0.075	0.038	0.075	0.042	0.038–0.3	0.089
	AmB	0.075–0.3	0.15	0.3	0.15	0.15–0.3	0.225
C. neoformans (21)	SPA-S-843	0.019–0.075	0.038	0.075	0.035	0.038–1.2	0.461
	AmB	0.038–0.3	0.15	0.3	0.15	0.075–1.2	0.263
S. cerevisiae (10)	SPA-S-843	0.002–0.019	0.009	0.019	0.009	0.019–0.6	0.15
	AmB	0.018–0.15	0.075	0.075	0.065	0.038–0.15	0.08

[a] AmB, amphotericin B.

Rimaroli C, & Bruzzese T. 1998. Antimicrobial Agents & Chenothera. 42 (11): 3012 -3013

TPU-0043 (Chainin)

- Pentaene macrolide
- Isolated form *Streptomyces* sp. TP-A0625
- Nuclear Magnetic Resonance revealed TPU-0043 is similar to chainin, that is isolated from *Chainia* sp.
- *In* vitro antifungal activity against *Aspergillus* sp. and against *Cryptococcus* sp.
- Not studied in any animal model
- Toxicity – not known



Chainin (**10**) R_1=H R_2=H
Isochainin (**11**) R_1=H R_2=H
12 R_1=OH R_2=H
13 R_1=H R_2=OH
14 R_1=OH R_2=OH

Igarashi Y et al. 2005. J Antibiot. 58(8): 523-525

Li Z et al. 1989. J Antibiot. 42(4): 577-584

YS-822A

- Tetraene macrolide antibiotic
- Isolated from culture filtrate of mutant strain H-8 of *Streptoverticillum eurocidicum* var. *asterocidicus* S-822
- Produces acute toxicity in mice (IV)

Fig. 2. Mutation schedule of *Streptoverticillium eurocidicum* var. *asterocidicus*.

UV: UV irradiation, PR: protoplast regeneration, NTG: nitrosoguanidine treatment.

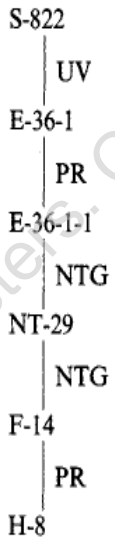


Table 5. Antifungal activity of YS-822A and other polyene macrolides.

Strains	MIC (μg/ml)		
	YS-822A	AMPH-B	NYS
Saccharomyces cerevisiae No. 6	3.12	0.78	0.78
Candida lypolytica ATCC 8862	12.5	0.78	3.12
C. pseudotropicalis IMC-F2	3.12	0.78	0.78
C. albicans IFO 1594	6.25	0.2	0.78
C. albicans IFO 1385	6.25	0.2	0.78
C. tropicalis IFO 0006	12.5	3.12	3.12
C. tropicalis TIMM 0315	12.5	1.56	3.12
Rhodotorula minuta	6.25	12.5	6.25
Hansenula anomala IFO 0138	6.25	0.2	0.78
Aspergillus niger	3.12	3.12	6.25
Fusarium roseum IAM 5010	6.25	3.12	3.12
Gibberella fujikuroi IFO 6349	25	100	25
Alternaria kikuchiana F2	3.12	1.56	1.56
Sporothrix schenckii IFO 8158	50	50	25
Trichophyton mentagrophytes IFO 5811	50	3.12	6.25
T. rubrum IFO 5808	50	1.56	1.56
Epidermophyton floccosum IFO 9045	1.56	0.2	0.39

Itoh A et al. 1990. J Antibiotics. 43(8):948-955

Conclusions

- Amphotericin B, Nystatin, and Natamycin is highly toxic; so the liposomal form helps in reducing the toxicity e.g. liposomal amphotericin B and liposomal nystatin

- More than 200 polyene macrolides have been identified; but could not be used in treatment due to their toxicity

- Variety of synthetic methodologies were developed to characterize the unique class of products from *Streptomyces*

- Biosynthesis of polyene macrolides have been generated through genetic manipulations, however the usefulness for treatemnt of these synthesized polyenes are being evaluated