Handout #5
Bio 329
2004

. Development and clinical spectrum of the various antifungals

Year developed	Generic drug	Type(s) of infection for which drug is principally used[1]				
		superficial[2]	dermatophytosis	candidosis	locally invasive	viscera; and system.
1903	Potassium iodide				Lymphangetic sporotrichosis	
1907	Whitfield's ointment	±	+			
1940	Undecylenic acid	Desonex	+			
1949/50	Nystatin (first useful antifungal antibiotic)			+		
1950	Hydroxystilbamidine					North American blastomycoses
1954/56	8-Hydroxyquinoline derivatives		+	±		
1957	Amphotericin B	Fungizone		+	+	+
1958	Griseofulvin		+			
1958/60	Pimaricin				Mycotic keratitis	
1961	Acrisorcin		+			
1963	Haloprogin		+	±		
3/68	Tolnaftate	Tinactin	+			
1963/68	Flucytosine			+	± *Cladiosporium* sp.	Cryptococcosis
1969/70	Miconazole	+ Micatin, Monistat	+	+	+	+
1969/70	Clotrimazole	+ Lotrimin	+	+		
1974/75	Econazole	+ Mycolex	+	+		
~1978	Ketoconazole	+ Nizoral	+	+	+	+

1 + = general effectiveness ± = limited effectiveness.
2 Includes pityriasis versicolor and tinea nigra palmaris.

1990 – Fluconazole (US) Diflucan
1994 – Itraconazole (US) Sporonox
1996 – Terbinafine (US) Lamisil

55a

also butoconazole nitrate = Femstat
terconazole = Terzole

Voriconazole – 2001 approval pending
Caspofungin – 2001 FDA approved – 1st lipopeptide approved in U.S. targets β1→3 glucan synthase

(1)

Table IV. The relationship between solubility of an antifungal and its marketed formulation(s)

Solubility in water	Topical dose forms	Intravenous dose forms	Oral dose forms
Soluble	Acrisorcin	Hydroxystilbamidine	Potassium iodide
Limited solubility	Clotrimazole		Ketoconazol Flucytosine Griseofulvin
Practically insoluble	Pimaricin, Nystatin, Miconazole, Econazole, Amphotericin B, Hydroxyquinoline, Whitfield's ointment, Undecyclenic acid, Tolnaftate, Haloprogin	Miconazole Amphotericin B	Itraconazole Fluconazole

Table 1. *Fungal infections.*

Site of infection	Disease	Fungus	Therapy
Lung	†Aspergillosis	*Aspergillus fumigatus*	Amphotericin B and certain imidazole drugs
	Blastomycosis Paracoccidioidomycosis	*Blastomyces dermatidis* *P. brasiliensis*	
	Coccidioidomycosis	*Coccidioides immitis*	
	Histoplasmosis	*Histoplasma capsulatum* *H. dubossi*	
Wounds	Chromomycoses	*Cladosporium carrionii* *Phialophora* spp.	Amphotericin B and certain imidazole drugs
	Mycetomas	16 spp. identified	
	Sporotrichosis	*Sporothrix schenkii*	
Skin and mucous membranes	Candidosis	*Candida albicans*	Polyenes, imidazole drugs and 5-fluorocytosine
	Dermatophytosis	*Epidermophyton* spp. *Microsporum* spp. *Trichophyton* spp.	Griseofulvin and imidazole drugs

†Opportunistic pathogens.

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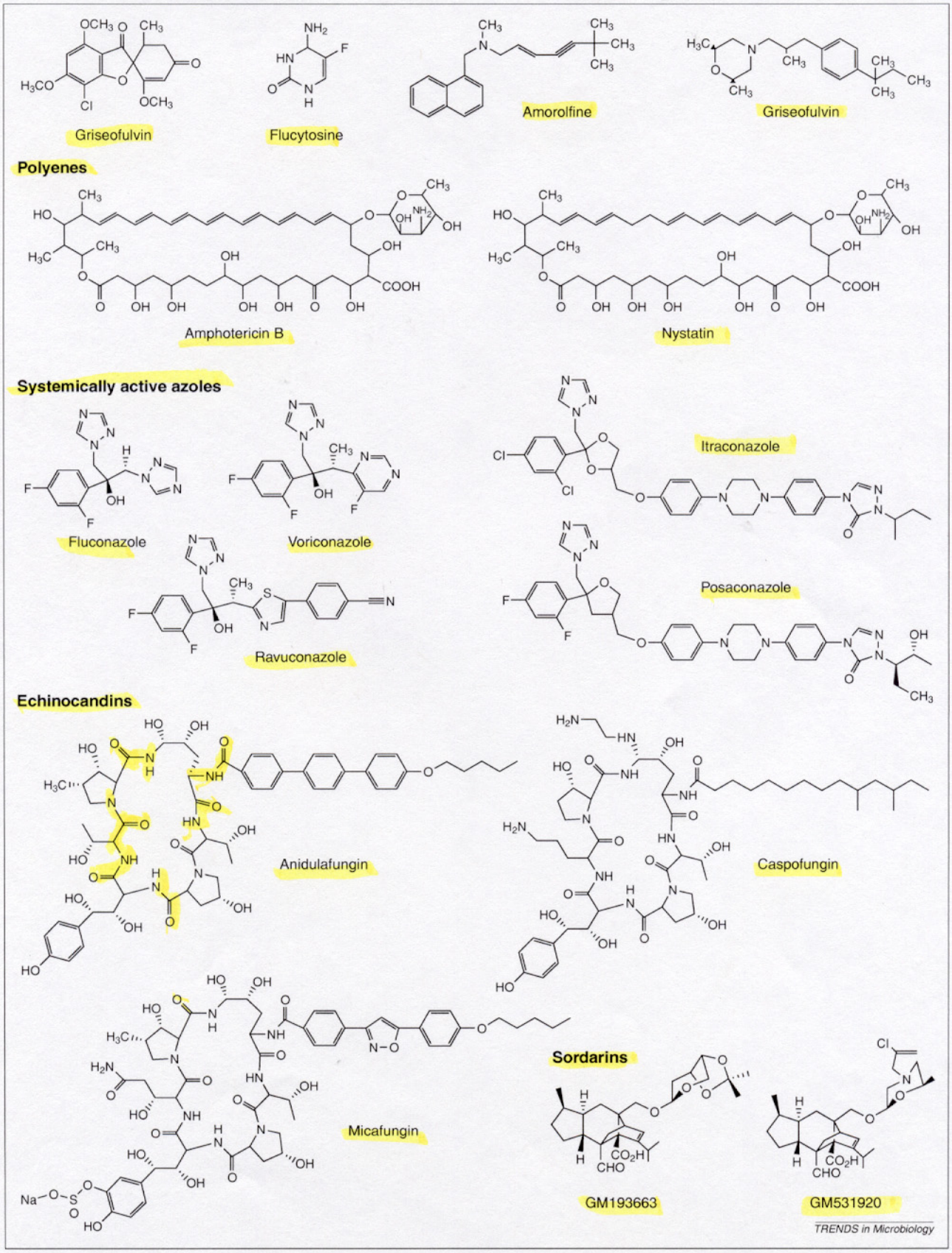
Griseofulvin
Flucytosine
Amorolfine
Griseofulvin
Polyenes
Amphotericin B
Nystatin
Systemically active azoles
Fluconazole
Voriconazole
Itraconazole
Ravuconazole
Posaconazole
Echinocandins
Anidulafungin
Caspofungin
Micafungin
Sordarins
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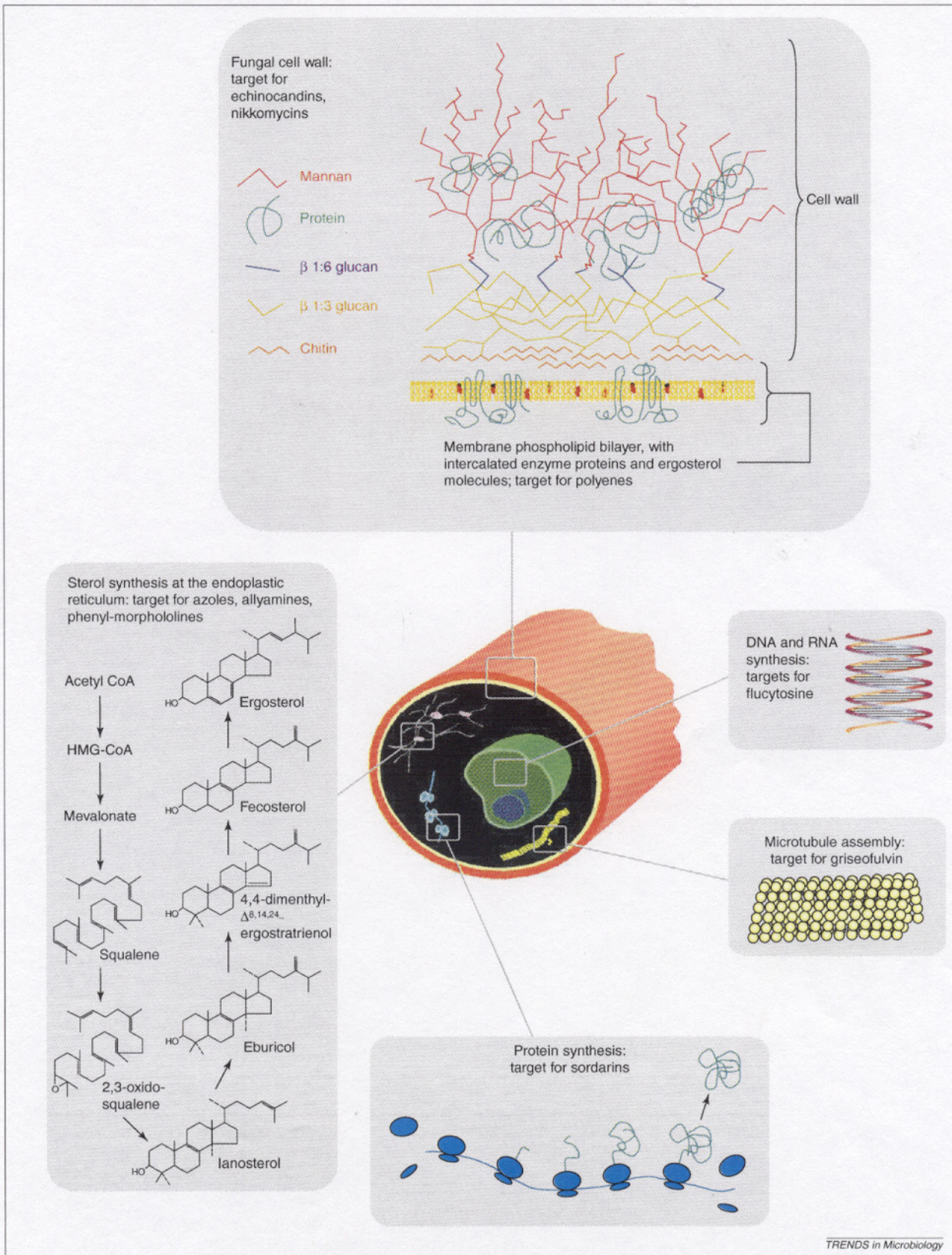
Fungal cell wall: target for echinocandins, nikkomycins
Mannan
Protein
β 1:6 glucan
β 1:3 glucan
Chitin
Cell wall
Membrane phospholipid bilayer, with intercalated enzyme proteins and ergosterol molecules; target for polyenes
Sterol synthesis at the endoplastic reticulum: target for azoles, allyamines, phenyl-morpholines
Acetyl CoA
HMG-CoA
Mevalonate
Squalene
2,3-oxido-squalene
Ergosterol
Fecosterol
4,4-dimenthyl-Δ8,14,24- ergostratrienol
Eburicol
Ianosterol
DNA and RNA synthesis: targets for flucytosine
Microtubule assembly: target for griseofulvin
Protein synthesis: target for sordarins
TRENDS in Microbiology

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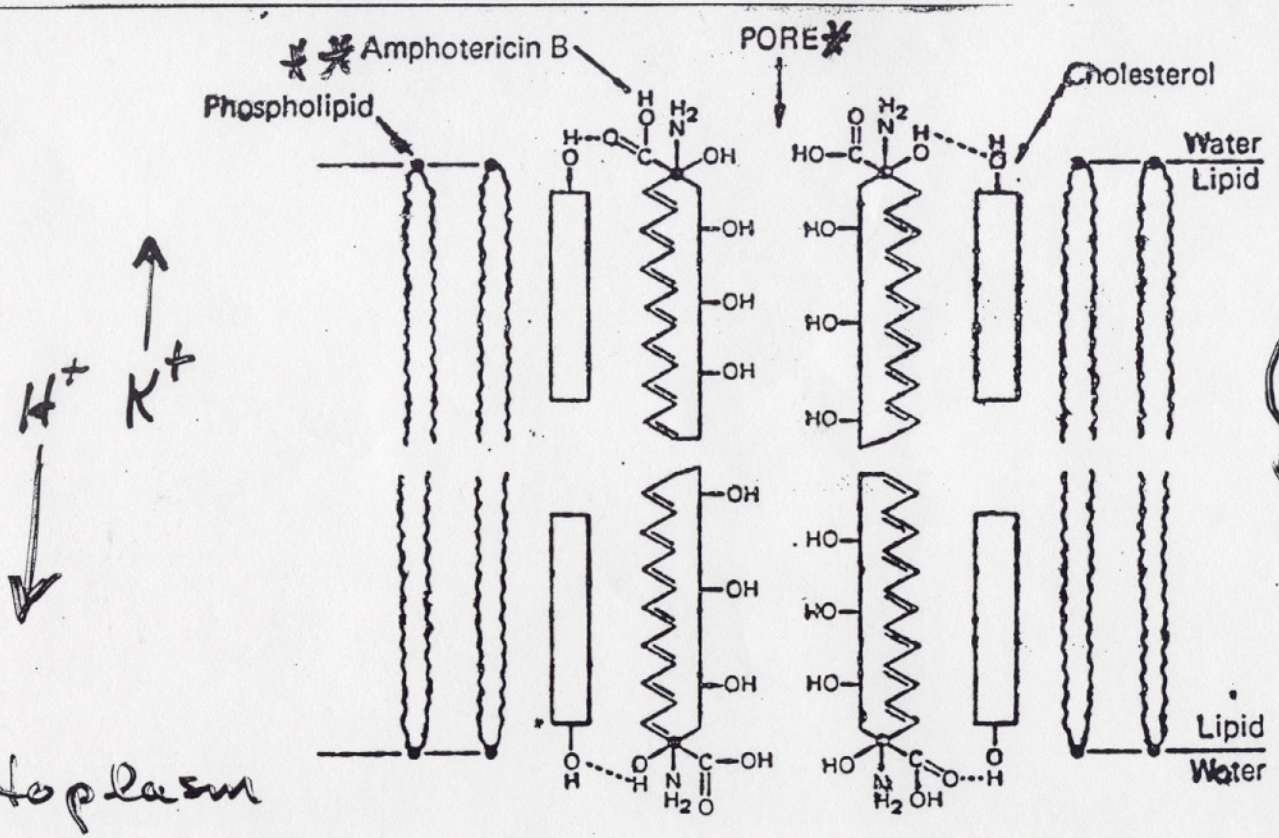

v. 1975 membrane

* 0.8 nm

** 8 molecules

Figure 13–1 A hypothetical model of a pore formed by amphotericin B in a lipd bilayer membrane. Considerable evidence supports the hypothesis that amphotericin B and nystatin can form pores in artificial lipid bilayer membranes. When the antibiotic is added to both sides of the membrane, the pores are quite anion selective. Since sucrose (radius = 5.2 Å) is not permitted to pass through, the radius of the pore is thought to be less than 4–5Å. The pore is formed by several* polyene molecules packed side by side in a cylinder formation. The principal interactions between the antibiotic and the membrane involve hydrophobic bonds between the lipophilic heptaene segment of the antibiotic and the sterols. The dashed lines represent possible hydrogen bonds. The solid circles oriented at the membrane surfaces represent the polar head groups of the phospholipids, and the wavy lines denote the hydrophobic fatty acid chains. In this configuration, amphotericin B is 20–24 Å long and extends into but does extend across the distance of the bilayer. The ion conductance of artificial bilayer membranes is markedly potentiated when the antibiotic is added to both sides, presumably because continuous pores passing from one membrane surface to the other can be readily formed.[21] (Adapted from Andreoli,[24] Figure 11)

* 0.8 nm?

Table 2. Therapy of choice for selected systemic mycoses. After [26].

Disease	Preferred agent	Alternative agents
Aspergillosis	Amphotericin B ± flucytosine or rifampicin	Itraconazole*
Blastomycosis	Amphotericin B or ketoconazole	Itraconazole* ?Fluconazole
Candidiasis (invasive or disseminated)	Amphotericin B ± flucytosine	?Ketoconazole ?Fluconazole
Chromomycosis	Flucytosine ± amphotericin B	Ketoconazole
Coccidioido-mycosis	Amphotericin B or ketoconazole	Fluconazole Miconazole Itraconazole*
Cryptococcosis	Amphotericin B and flucytosine	Fluconazole Itraconazole*
Histoplasmosis	Amphotericin B or ketoconazole	Itraconazole* ?Fluconazole
Mucormycosis	Amphotericin B	None
Paracoccidioido-mycosis	Ketoconazole or amphotericin B	Sulphonamide Miconazole Itraconazole*
Pseudoallescher-iasis	Miconazole	Ketoconazole
Sporotrichosis (extracutaneous)	Amphotericin B	Itraconazole* ?Fluconazole

*Investigational drug in certain countries.

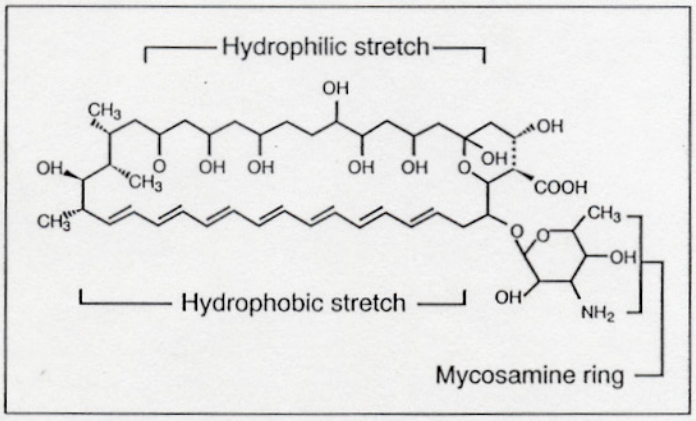

Figure 1. Structural features of amphotericin B. After [29].

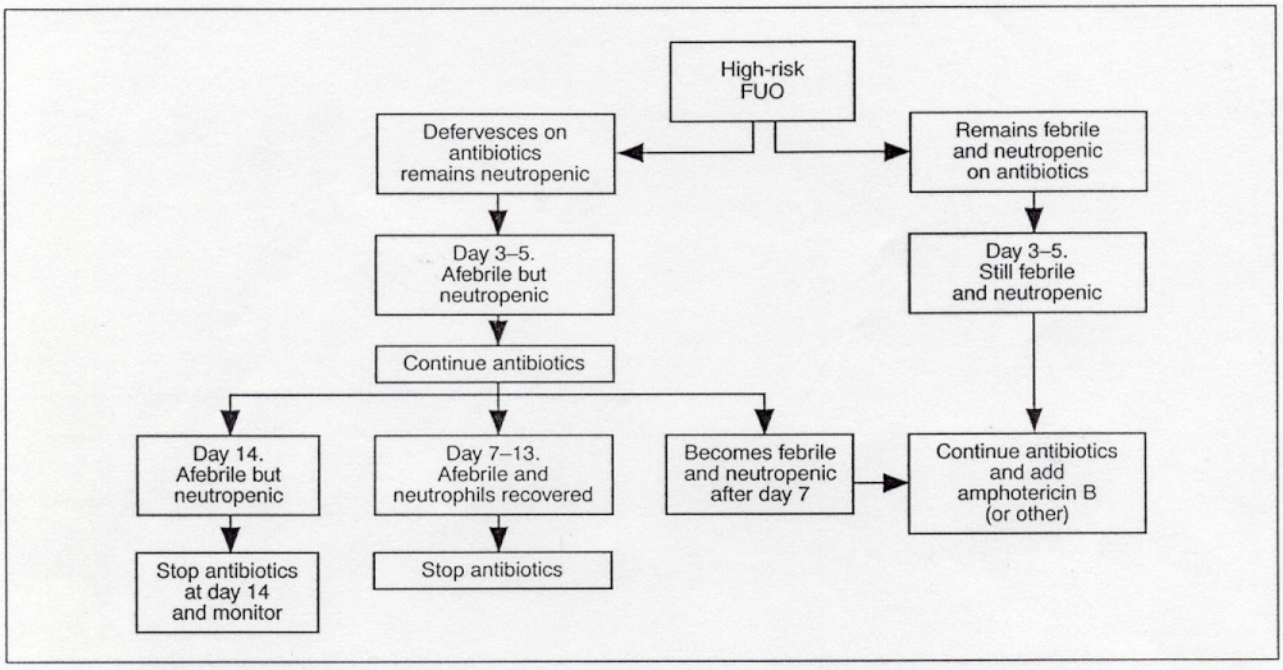

Figure 3. Algorithm for the initial management of febrile neutropenic patients. Adapted from [15].

3. RATIONAL DEVELOPMENT OF ABELCET®

(i) Lipid-based engineering

The usefulness of lipid-based engineering such as liposomes, as targeted carriers of pharmacological agents stems from their water-lipid structure and particulate nature. Liposomes are primarily composed of biodegradable, reusable phospholipids which are structurally characterised by a hydrophilic head attached to a hydrophobic tail. When placed in water, they arrange themselves spontaneously into bilayer structures so that the hydrophobic tails are shielded from the water by hydrophilic heads. As it is thermodynamically unfavourable to have hydrophobic edges adjacent to water, the bilayer sheets form a closed system with water both inside and outside the bilayer (Figure 4) [56].

Water-soluble substances such as certain drugs, enzymes or genes, can be entrapped within the aqueous spaces of the bilayer, whereas fat-soluble entities such as polyene antibiotics and other lipid soluble drugs can be incorporated into the lipid layers [56]. Preparation techniques allowing control over size, lamellarity, trapped aqueous volume, and solute distribution in the final emulsions have led to a rapid expansion in this field [57].

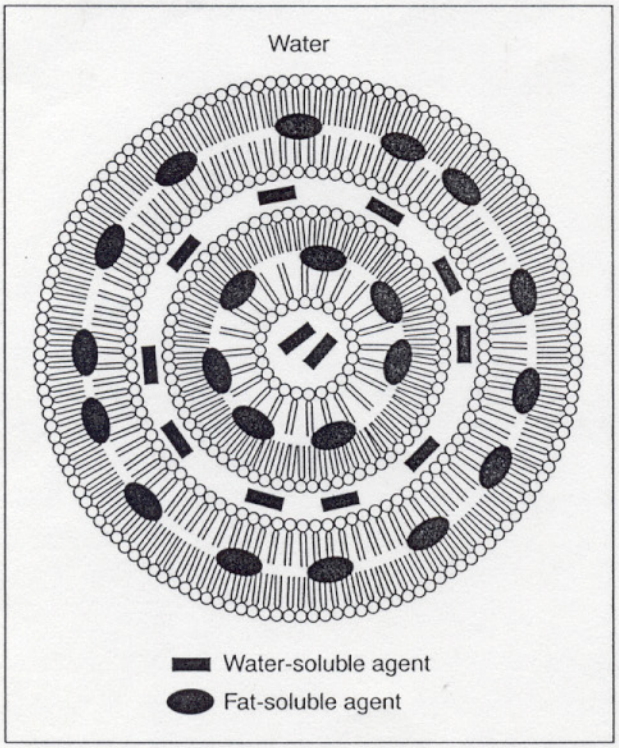

Figure 4. Schematic diagram of fat- and water-soluble agents encapsulated in a liposome. After [56].

Lipid-based engineering also offers an adaptable means of redesigning the properties of pharmaceuticals. Pharmaceutical agents designed in this way have unique pharmacokinetic and pharmacodynamic characteristics. The way lipid-based drugs are distributed throughout the body differs from that of conventional drugs. Studies have shown that lipid-based drugs appear to be retained selectively in the organs of the reticuloendothelial system (RES) such as the liver, spleen, lungs, lymph nodes, and to a lesser extent bone marrow. One explanation for this is the phagocytic nature of the system – lipid-rich particles can be ingested by phagocytic cells such as monocytes, providing a mechanism for targeting to sites of infection and a means of intracellular drug delivery. The tendency of lipid-based drugs to accumulate at sites of infection, inflammation and neoplasms is probably due to ingestion and delivery by phagocytic cells. In addition, it may be due to the disease process enlarging the spaces between the endothelial cells lining local capillaries at those sites, permitting the extravasation of liposomal or lipid-based drugs [56].

Lipid-based engineering of pharmacokinetics/pharmacodynamics has been described for a variety of biologically active compounds including polyene antifungals, anthracyclines, aminoglycoside antibiotics and prostaglandins [56]. Although redesigning pharmaceuticals in this way can attenuate toxicities of associated drugs, efficacy of the treatment should not be compromised and should be thoroughly investigated [58]. For example, a study by Pahls and Schaffner, which compared the antifungal activity of a unilamellar liposomal preparation of amphotericin B with conventional amphotericin B *in vitro* and in models of systemic and localised candidiasis in immunosuppressed mice, reported that the liposomal amphotericin B preparation had 4–8 times less antifungal activity than conventional amphotericin B [58].

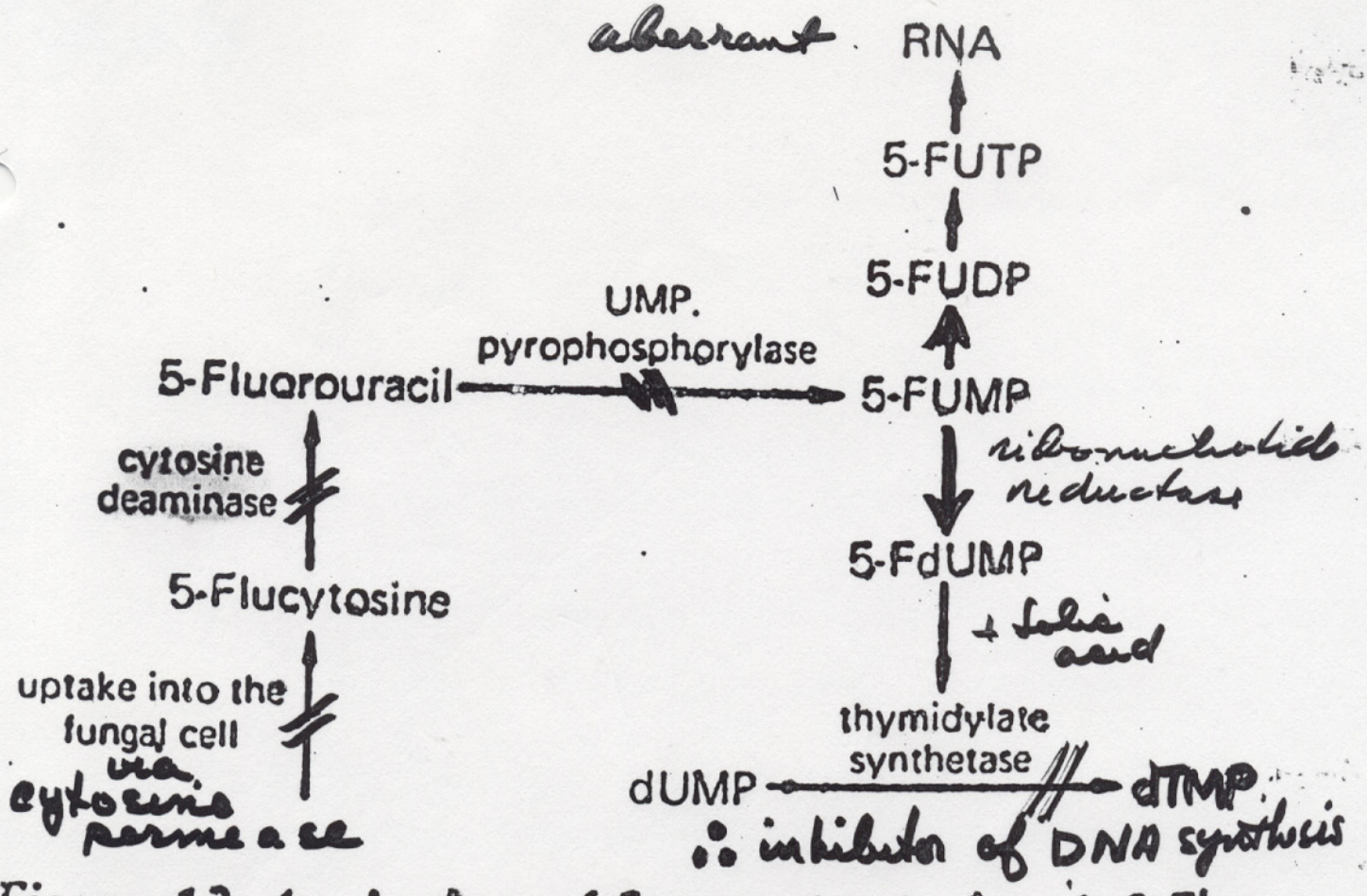

Figure 13–4 Action of flucytosine in fungi. 5-Flucytosine is transported into the fungal cell where it is deaminated to 5-fluorouracil (5-FU). The 5-FU is then converted to 5-fluorouracil-ribose monophosphate (5-FUMP) which can either be converted to 5-FUTP and incorporated into RNA or be converted by ribonucleotide reductase to 5-FdUMP which is a potent inhibitor of thymidylate synthetase. The arrows with break marks represent those reactions that have been shown to be absent in various flucytosine-resistant fungi.

Rouch → ancoben

Biochemical basis for the activity and selectivity of oral antifungal drugs

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Summary

The ergosterol biosynthesis-inhibiting (EBI) antifungals constitute the most important group of compounds developed for the control of fungal diseases in man. Currently, representatives of two classes of EBI antifungals are available: the squalene epoxidase inhibitors and those that interfere with cytochrome P450-dependent ergosterol synthesis.

The allylamines (eg, terbinafine) inhibit squalene epoxidase in sensitive fungi, *Trichophyton mentagrophytes* being the most sensitive species.

The most important developments have come from the introduction of the N-substituted imidazoles and triazoles, the so-called azole antifungals. Most of the currently available imidazoles (eg, miconazole, clotrimazole, econazole) and the triazole derivative terconazole are mainly for topical treatment. Ketoconazole was the first azole derivative orally active against yeasts, dermatophytes and dimorphic fungi. The new triazole, itraconazole, appears to be among the most promising orally active systemic agents. All the azole antifungals inhibit the cytochrome P450-dependent, 14α-demethylase, a key enzyme in the synthesis of ergosterol, the main sterol in most fungal cells. Of all the azoles tested, itraconazole shows the highest affinity for the cytochrome P450 involved. It is about three and ten times more active *in vitro* than miconazole and the bis-triazole, fluconazole, respectively. Itraconazole's high affinity for the fungal P450 originates from its triazole group as well as from the non-ligating lipophilic tail.

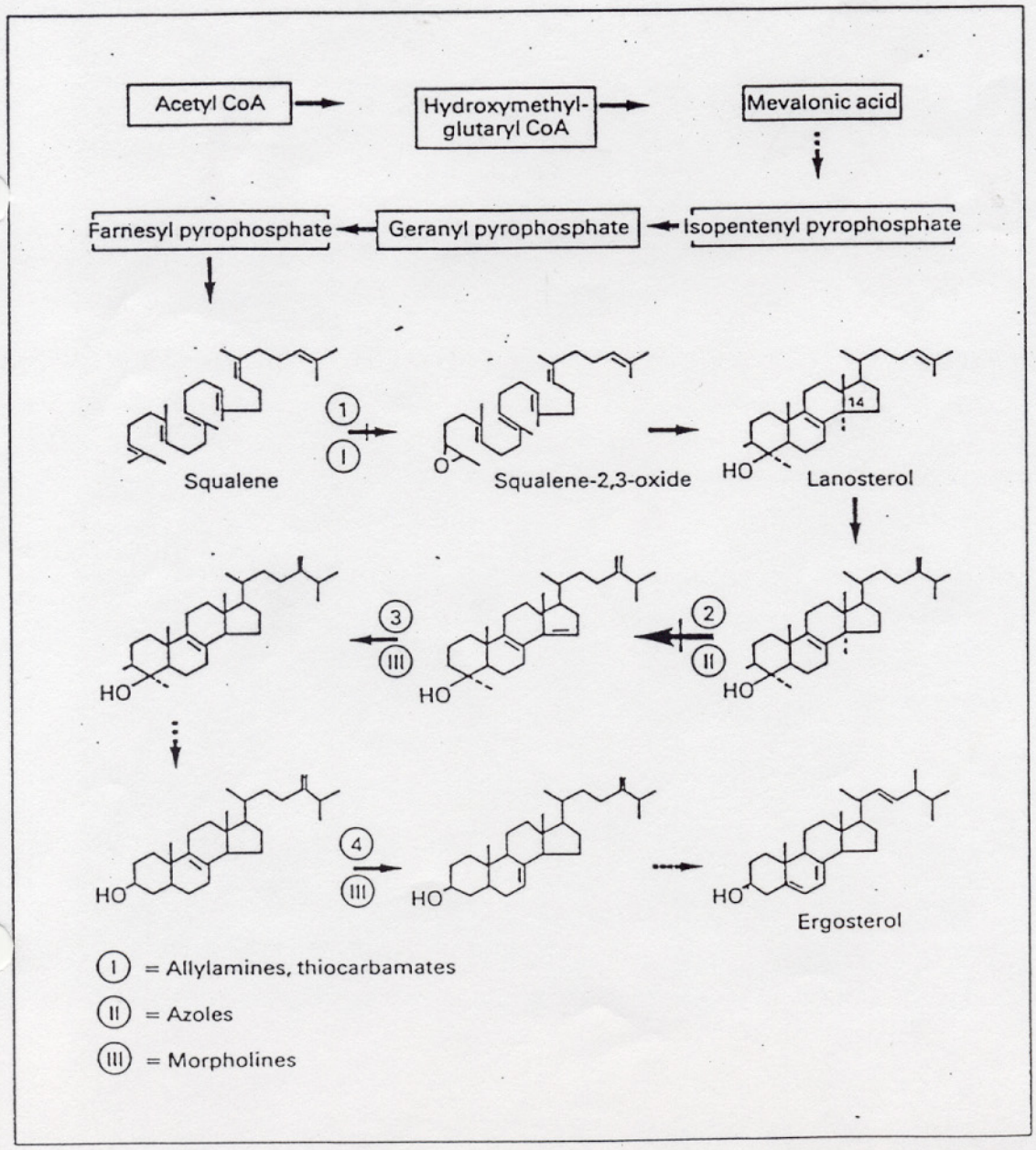

Fig 1. The fungal ergosterol biosynthetic pathway, showing the steps inhibited by the main antifungal agents.

Table 1: Structure, classification, probable mechanisms of action, indications for clinical use, and principal toxic effects of antifungal agents used in treatment of deep mycoses.

Antifungal agent	Chemical structure	Probable mechanism(s) of action	Indications for treatment of deep mycoses and/or etiologic fungi	Principal toxic effects
Amphotericin B	polyene macrolide	combination with sterols, especially ergosterol in the cytoplasmic membrane to establish pores in cell membrane; also oxidative membrane damage	*Aspergillus* spp., *Candida* spp., *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Trichosporon beigelii*, *Fusarium* spp., *Bipolaris*, *Exserohilum*	nephrotoxicity: azotemia, renal tubular acidosis, hypokalemia, hypomagnesemia, nephrocalcinosis bone marrow suppression fever and shaking chills during infusion thrombophlebitis
Flucytosine	fluorinated pyrimidine	5-FC → 5-FU → 5 FUMP → 5-FUdMP + 5-FUTP 5-FUdMP: inhibition of fungal thymidilate synthetase and DNA synthesis; 5-FUTP: inhibition of RNA processing	used in combination with amphotericin B for deep mycoses due to *Cryptococcus neoformans*, some dematiaceous hyphomycetes, and *Candida* spp.	bone marrow suppression diarrhea hepatotoxicity cerebellar defects
Miconazole	N-substituted imidazole	inhibition of synthesis of membrane sterols at the level of oxidative ^{14}C-demethylation through inhibition of yeast cytochrome P-450-dependent enzymes	*Pseudallescheria boydii*	pruritis, headache, thrombophlebitis headache thrombophlebitis hepatotoxicity
Ketoconazole	N-substituted imidazole	similar to that of miconazole	non-meningeal infections due to *Paracoccidioides braziliensis*, chronic cavitary histoplasmosis, disseminated histoplasmosis in clinically stable non-immunosuppressed patients, non-meningeal blastomycosis and certain forms of coccidioidomycosis (cutaneous lesions, draining sinus tracts, soft tissue abscesses, non-cavitary pulmonary infiltrates, synovitis and osteomyelitis)	nausea, vomiting, anorexia, hepatoxicity: usually transient; rarely icteric endocrinologic; gynecomastia; diminished libido; rarely, adrenal insufficiency
Itraconazole	N-substituted triazole	similar to that of miconazole	investigational: indications similar to those for ketoconazole with possible addition of sporotrichosis and selected conditions of aspergillosis	investigational: less gastrointestinal toxicity than ketoconazole; possible aldosterone-like effect
Fluconazole	N-substituted bis-triazole	similar to that of miconazole	investigational: cryptococcal meningoencephalitis	investigational: infrequent nausea; infrequent hepatotoxicity (asymptomatic transaminase elevation)

5-FC: 5-fluorocystosine (flucytosine).
5-FU: 5-fluorouracil.
5-FUMP: 5-fluoruridine monophosphate.
5-FdUMP: 5-fluorodeoxyuridine monophosphate.
5-FUTP: 5-fluorouridine triphosphate.