Ionophores

Ionophores

An ionophore is a lipid-soluble molecule usually synthesized by microorganisms to transport ions across the lipid bilayer of the cell membrane.

The two broad classifications of ionophores are:

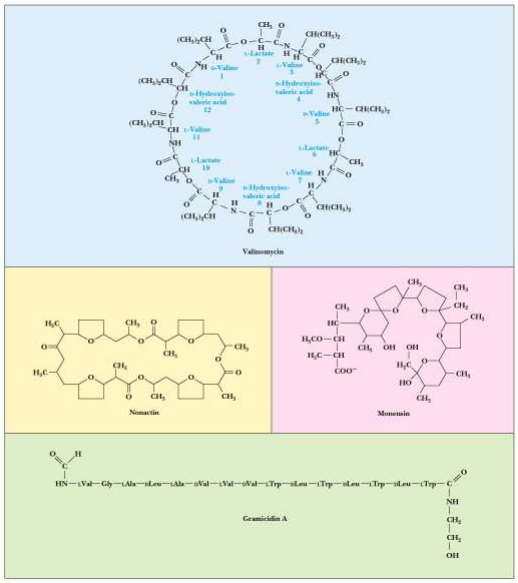
Chemical compounds (mobile ion carriers) that bind to a particular ion, shielding its charge from the surrounding environment, and thus facilitating its crossing of the hydrophobic interior of the lipid membrane : valinomycin

Channel formers that introduce a hydrophilic pore into the membrane, allowing ions to pass through while avoiding contact with the membrane's hydrophobic interior : gramicidin

Ionophore Antibiotics

Mobile carrier or pore (channel)

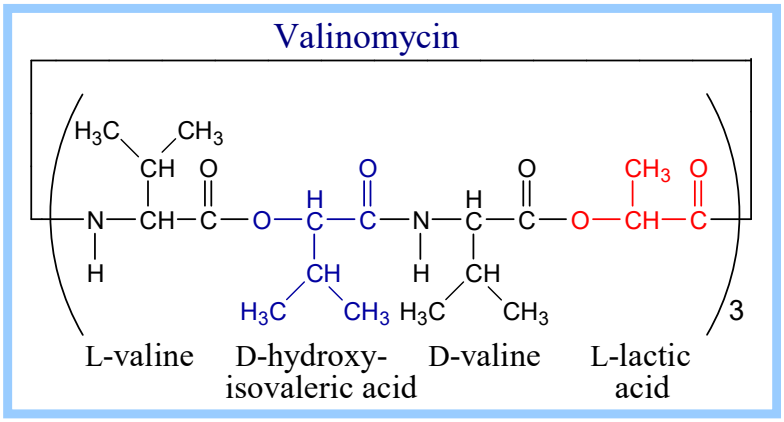
- **How to distinguish? Temperature!**
- **Pores will not be greatly affected by temperature, so transport rates are approximately constant over large temperature ranges**
- **Carriers depend on the fluidity of the membrane, so transport rates are highly sensitive to temperature, especially near the phase transition of the membrane lipids**



Valinomycin

A classic mobile carrier

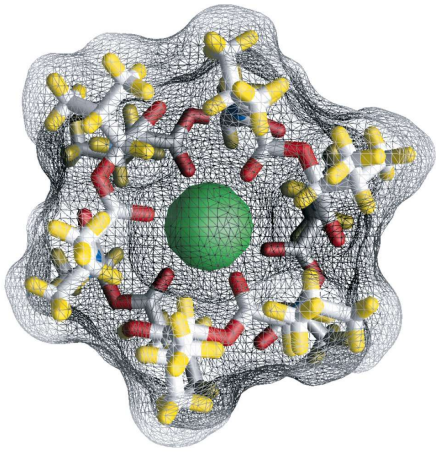
- A depsipeptide - a molecule with both peptide and ester bonds
- Valinomycin is a dodecadepsipeptide
- The structure places several carbonyl oxygens in the center of the ring structure
- Potassium and other ions coordinate the oxygens
- Valinomycin-potassium complex diffuses freely and rapid across membranes



Valinomycin is a **carrier** for K^+.

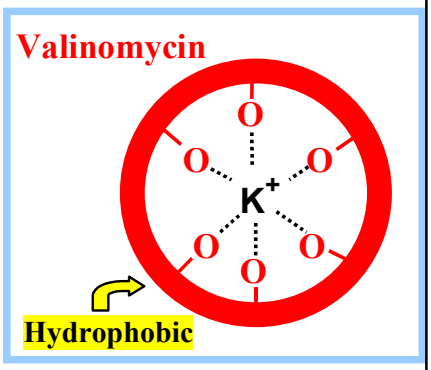
It is a **circular** molecule, made up of 3 repeats of the sequence shown above.

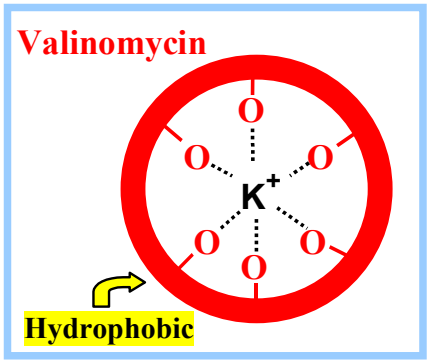
Passive Ionophore Carrier: Valinomycin



Puckering of the ring, stabilized by H-bonds, allows valinomycin to closely surround a single unhydrated K^+ ion.

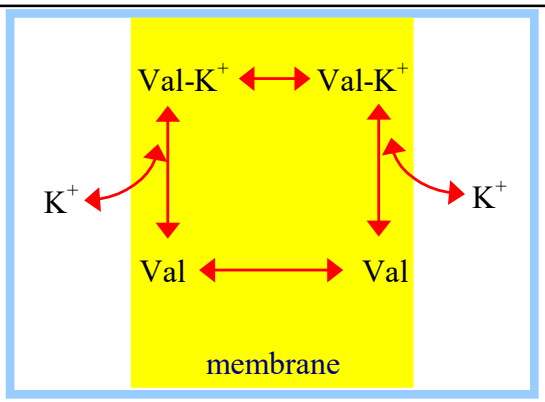
Six oxygen atoms of the ionophore interact with the bound K^+, replacing O atoms of waters of hydration.





Whereas the interior of the valinomycin-K^+ complex is polar, the **surface** of the complex is **hydrophobic**.

This allows valinomycin to enter the lipid core of the bilayer, to solubilize K^+ within this hydrophobic milieu.



Valinomycin is a **passive carrier** for K^+. It can bind or release K^+ when it encounters the membrane surface.

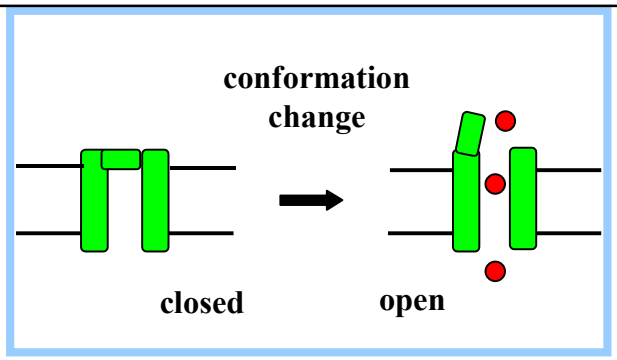
Valinomycin can catalyze **net K^+ transport** because it can translocate either in the complexed or uncomplexed state.

The direction of net flux depends on the electrochemical K^+ gradient.

Selectivity of Valinomycin

- **K^+ and Rb^+ bind tightly, but affinities for Na^+ and Li^+ are about a thousand-fold lower**
- **Radius of the ions is one consideration**
- **Hydration is another (solvation energies)**
- **It "costs more" energy to desolvate Na^+ and Li^+ than K^+**

Ion Channels



Channels cycle between open & closed conformations.

When open, a channel provides a **continuous pathway through the bilayer**, allowing flux of many ions.

Gramicidin is an example of a channel.

Gramicidin

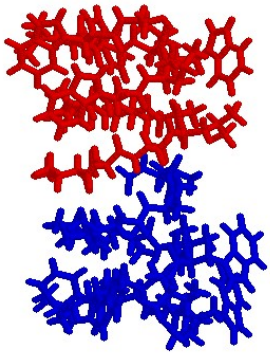
A classic channel ionophore

- **Linear 15-residue peptide - alternating D & L**
- **Structure in organic solvents is double helical**
- **Structure in water is end-to-end helical dimer**
- **Unusual helix - 6.3 residues per turn with a central hole - 0.4 nm or 4 A diameter**
- **Ions migrate through the central pore**

Gramicidin is an unusual peptide, with alternating D & L amino acids.

In lipid bilayer membranes, gramicidin **dimerizes** & folds as a right-handed **β-helix**.

The dimer just spans the bilayer.



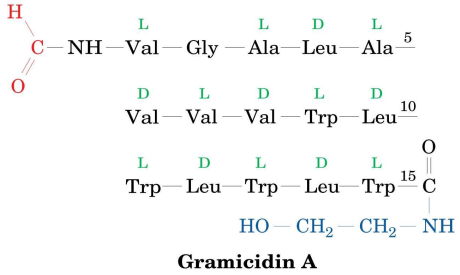
Gramicidin dimer (PDB file 1MAG)

Primary structure of gramicidin (A):

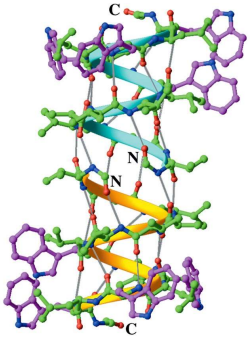
HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OH$

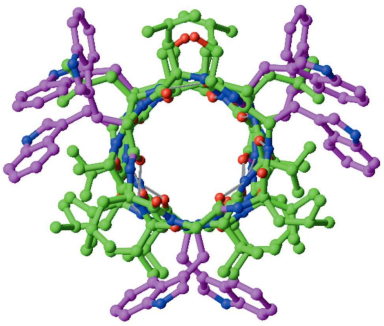
Note: The amino acids are all hydrophobic; both peptide ends are modified (blocked).

Passive Channel



Gramicidin A

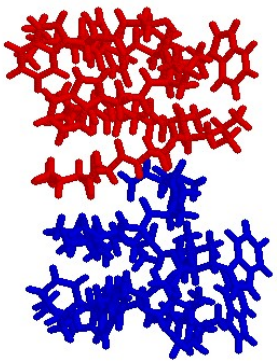




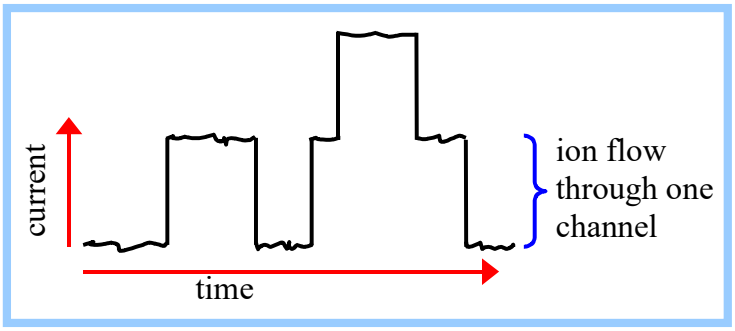
The **outer surface** of the gramicidin dimer, which interacts with the core of the lipid bilayer, is **hydrophobic**.

Ions pass through the more **polar lumen** of the helix.

Ion flow through individual gramicidin channels can be observed if a small number of gramicidin molecules is present in a lipid bilayer separating 2 compartments containing salt solutions.



Gramicidin dimer
(PDB file 1MAG)

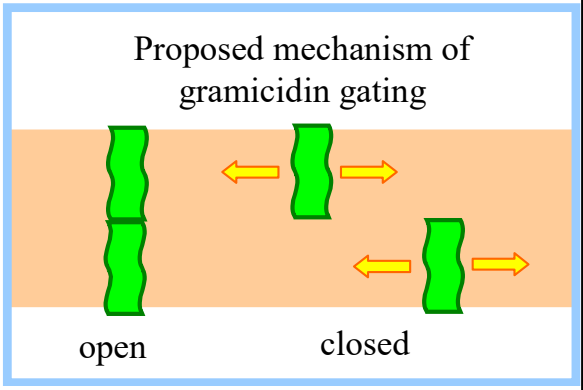


With voltage clamped at some value, **current** (ion flow through the membrane) **fluctuates**.

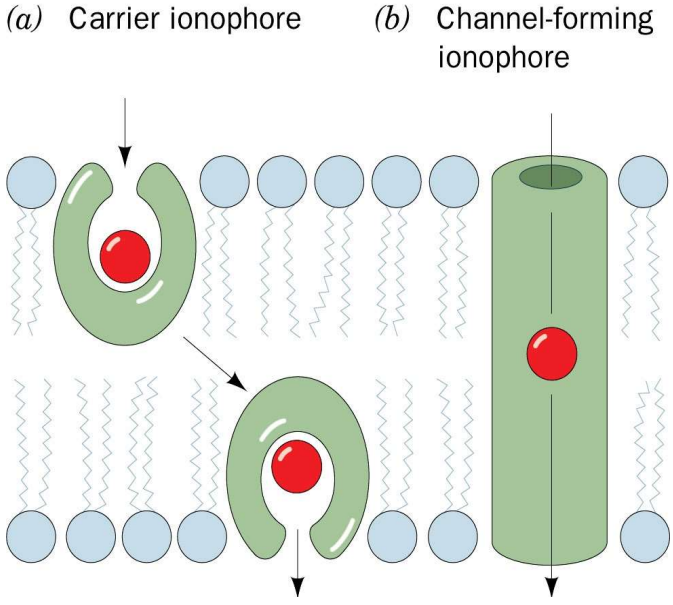
Each fluctuation, attributed to opening or closing of one channel, is the same magnitude.

The current increment corresponds to current flow through a single channel.

Gating (opening & closing) of a gramicidin channel is thought to involve reversible **dimerization**.



An open channel forms when two gramicidin molecules join end to end to span the membrane.



Passive Ionophore Transport

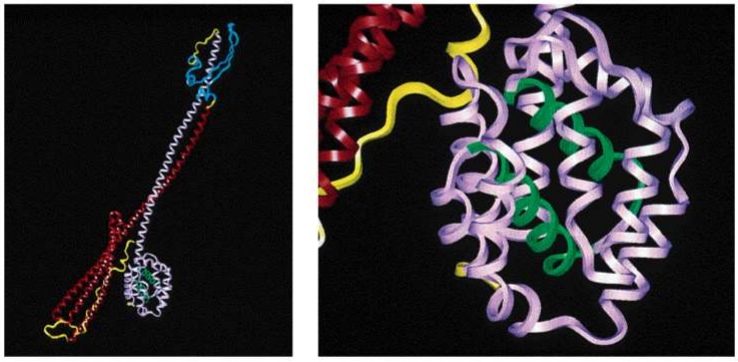
The Pore-Forming Toxins

- Lethal molecules produced by many organisms
- They insert themselves into the host cell plasma membrane
- They kill by collapsing ion gradients, facilitating entry by toxic agents, or introducing a harmful catalytic activity

Colicins

- Produced by *E. coli*
- Inhibit growth of other bacteria (even other strains of *E. coli*)
- Single colicin molecule can kill a host!
- Three domains: translocation (T), receptor-binding (R), and channel-forming (C)

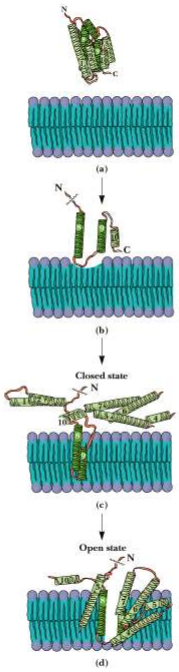
Garrett & Grisham: Biochemistry, 2/e
Figure 10.30



Saunders College Publishing

Clues to Channel Formation

- C-domain: 10-helix bundle, with H8 and H9 forming a hydrophobic hairpin
- Other helices amphipathic
- H8 and H9 insert, with others played on the membrane surface
- A transmembrane potential causes the amphipathic helices to insert!



Other Pore-Forming Toxins

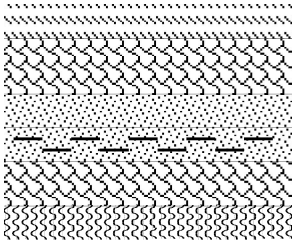
- **Delta endotoxin** also possesses a helix-bundle and may work the same way
- There are other mechanisms at work in other toxins
- **Hemolysin** from *Staphylococcus aureus* forms a symmetrical pore
- **Aerolysin** may form a heptameric pore - with each monomer providing 3 beta strands to a membrane-spanning barrel

Biochemical Targets for Antifungal Chemotherapy

Fungal cells are complex organisms **that share many biochemical targets** with other eukaryotic cells. Therefore, agents that interact with fungal targets not found in eukaryotic cells are needed.

The fungal **cell wall** is a unique organelle that fulfills the criteria for selective toxicity.

Fungal cell wall differs greatly from bacterial cell wall. Therefore, fungi are unaffected by antibacterial cell wall inhibitors such as **β-lactams** and **vancomycin**.



FIBRILLAR LAYER
MANNOPROTEIN
β-GLUCAN
β-GLUCAN, CHITIN
MANNOPROTEIN
PLASMA MEMBRANE

Biochemical Targets for Antifungal Chemotherapy

Arrangement of the biomolecular components of the cell wall accounts for the individual identity of the organism. Although, each organism has a different biochemical composition, their gross cell wall structure is similar.

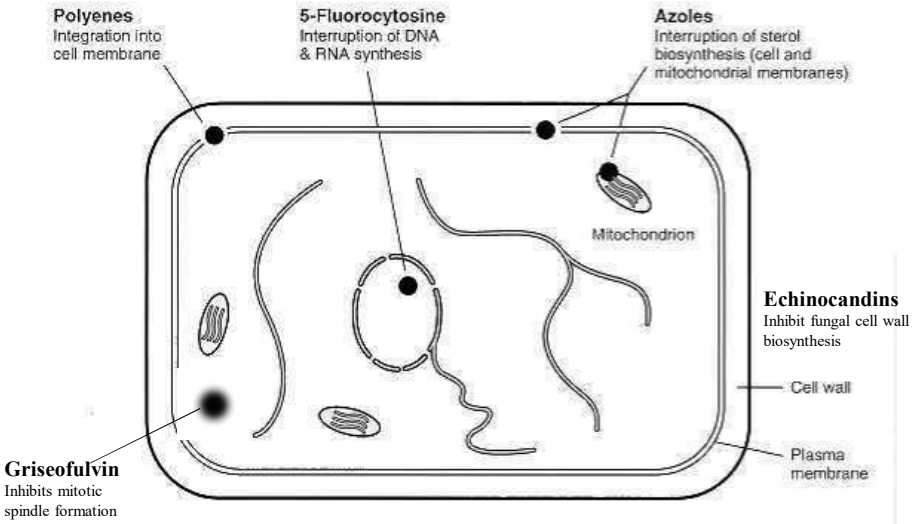
Antifungal agents targeted towards:

Inhibition of fungal cell wall synthesis – **caspofungin** is a β-glucan synthesis inhibitor

Inhibition of fungal cell membrane synthesis – ergosterol is the target (cell membranes of fungi and mammals contain different sterols): **polyenes**, azoles, triazoles, alkylamines

Inhibition of cell division – microtubule effects: **griseofulvin**; DNA: **flucytosine**.

Antifungal Agents- Sites of action



Antifungal Agents

Polyene:

These drugs interact with ergosterol in the fungal cell membrane and form pores

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

Antifungal Agents

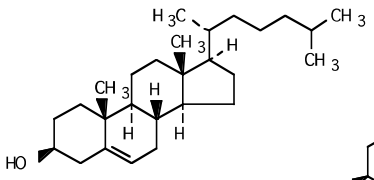
Polyene:

The number of conjugated double bonds correlates directly with antifungal activity in vitro and inversely with the degree of toxicity to mammalian cells.
They are unstable, only slightly soluble, and poorly absorbed when taken orally.

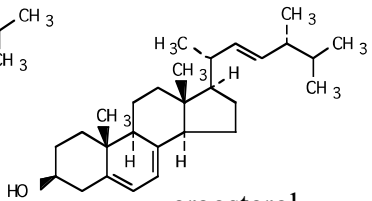
- **Amphotericin B**
- **Nystatin**
- **(Natamycin) Pimaricin**

Mechanism of Action of Polyenes

Polyenes bind to fungal membrane sterols. The selective effect is achieved because the sterol in highest concentration is ergosterol and polyenes have a high affinity for ergosterol. They insert into the membrane and disrupt membrane function. The membranes become leaky.

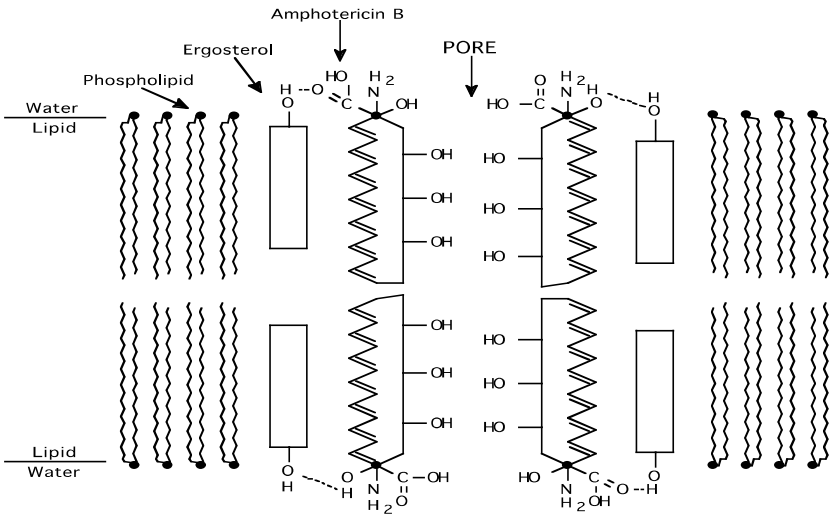


cholesterol



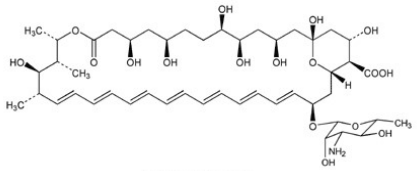
ergosterol

Mechanism of Action of Polyenes



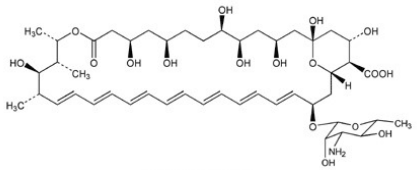
Amphotericin B

Amphotericin B, is produced by *Streptomyces nodosus*. Discovered in 1956 has been for 30 years the main available drug to control serious fungal infections. Amp. B is indicated for treatment of severe, potentially life threatening fungal infections.



amphotericin B

Amphotericin B



amphotericin B

Unfortunately, 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.

Amphotericin B

Disposition

Amp. B is highly bound to cholesterol-lipoprotein and has a plasma T1/2 of about 1 day and 1-2 weeks from tissues. It is excreted in urine over a long time.

Penetration into the CNS is poor. However, for fungal infections of the CNS, amphotericin B is mixed with cerebrospinal fluid (CSF) that is obtained from a spinal tap. The solution of amphotericin is then reinjected through the tap.

Amphotericin B

Adverse Effects:

1) **Reactions on infusion** - headache, fever, chills, anorexia, vomiting, muscle and joint pain. Pain at site of injection and thrombophlebitis are frequent

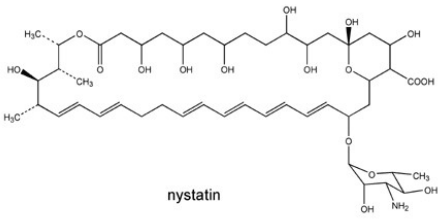
2) **Nephrotoxicity** - chronic renal toxicity in up to 80% of patients taking the drug for prolonged periods.

3) **Hematologic** - hemolytic anemia due to effects on RBC membrane.

4) **Other** less common reactions - cardiac, convulsions, neuropathy, hearing loss, allergic, etc.

Nystatin

Nystatin :
Isolated from *Streptomyces noursei* in 1951. A conjugated tetraene, is the first clinically useful polyene antifungal antibiotic. Available in oral tablets, powder for suspension. This polyene is used for local therapy only .



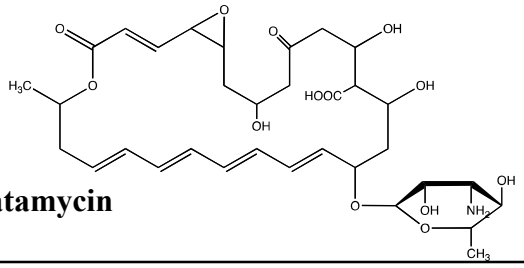
nystatin

No significant adverse effects with these uses. Combined with tetracycline to prevent monilial overgrowth caused by the destruction of bacterial microflora of the intestine during tetracycline therapy.

Natamycin

Natamycin (Pimaricin; Natacyn)

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.
Natamycine supplied as a 5% ophthalmic suspension intended for the treatment of fungal conjunctivitis, blepharitis and keratitis.



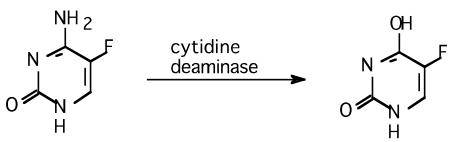
Natamycin

Nucleoside Antifungals

Orally active antifungal with a very narrow spectrum of activity

Flucytosine was synthesized in 1957 as an antitumor agent. It was inactive but it was found to have antifungal activity. Drug enters fungal cell through active transport on ATPases that normally transport pyrimidines. Once inside cells, fungal cytosine deaminase convert the drug to active 5-fluorouracl (5FU) which is incorporated into RNA causing faulty RNA synthesis. It is also is a strong, non-competitive inhibitor of thymidylate synthesis. Mammalian cells do not contain cytosine deaminase.

Flucytosine (5-Fluorocytosine)



Flucytosine 5-Fluorouracil

Nucleoside Antifungals

Resistance develops rapidly and occurs on many levels e.g. transport into the cell and cytosine deaminase steps. After a few dosing intervals the drug is essentially useless. To avoid rapid resistance, combination with Amphotericin B, and the combination is synergistic. It is also synergistic with itraconazole and fluconazole. Amphotericin B damaged membranes are thought to allow better entry of flucytosine.

Used (with Amp. B) for Cryptococcal meningitis, systemic Candida infections, and some other systemic fungal infections.

Adverse Effects
1)GI upset - very common.

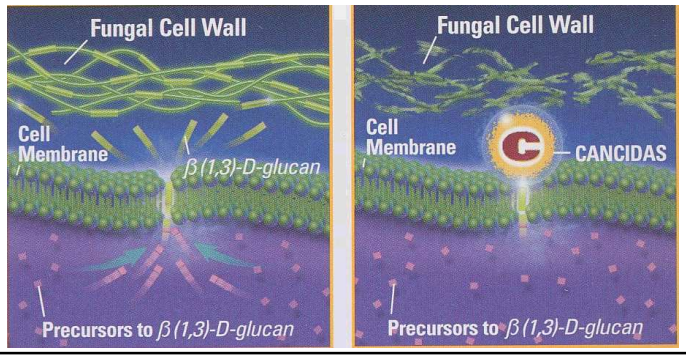
2)Hepatic - 5% have increased transaminases.

3)Hematologic - anemia, leukopenia, thrombocytopenia; this is the major complication of therapy and may be due to low levels of 5-FU circulating.

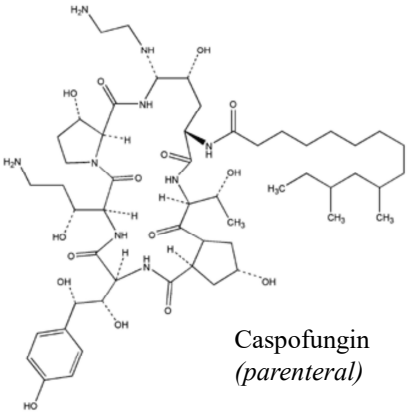
4)adverse effects seen when plasma levels reach >100 mcg/ml

Cell wall inhibitors

Echinocandins, a group of cyclic peptides with long lipophillic sidechains. They interfere with cell wall biosynthesis through inhibition of the enzyme β-1,3-glucan synthase. Reduction of in the glucan content weakens the cell wall and leads to rupture of fungal cells.

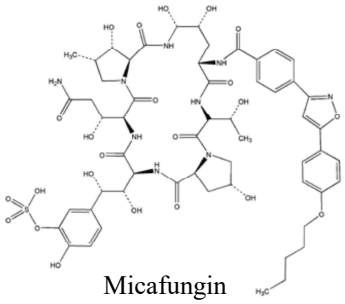


Echinocandins

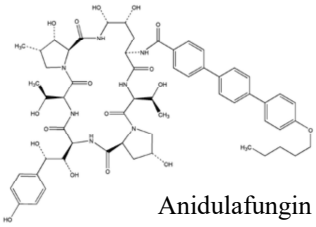


Caspofungin
(parenteral)

approved for invasive aspergillosis in patients refractory to or intolerant of other therapies . IV use only



Micafungin



Anidulafungin

Miscellaneous Antifungals

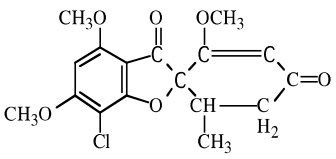
Griseofulvin

Antifungal antibiotic produced from *Penicillium griseofulvin*. Effects on microtubules to inhibit cell division

Therapy must continue until new tissue replaces old diseased tissue. When given orally, plasma-borne griseofulvin becomes incorporated into keratin precursor cells and ultimately into keratin which cannot then support fungal growth.

Griseofulvin is mainly effective on dermatophytes

Headache is a common adverse effect. May cause aplastic anemia. Being gradually replaced by newer agents.



Antifungal agents under development

All inhibitors of fungal cell walls

a) Other β-1,3 glucan synthetase inhibitors:
Papulacandins – glycolipid antifungal produced by Papularia sp.

b) Chitin Synthase inhibitors: Polyoxins and Nikkomycins– nucleoside peptides

c) Mannan binding antifungals: Pradimicins and benanomicins