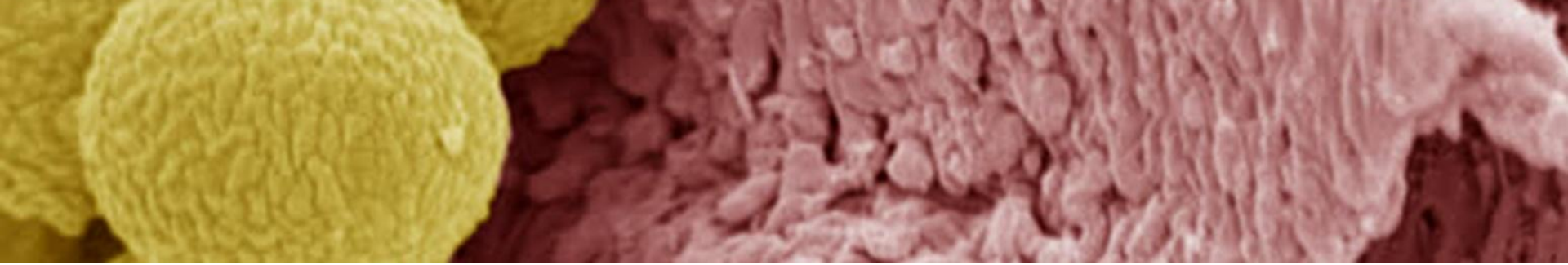

Antifungal agents

Introduction

- The development of chemotherapy to treat fungal infections is much lagged behind those to treat bacterial infections. This is because fungal infections are:
  - Usually superficial to skin and mucosal membranes
  - Rarely lethal
  - Easily coped by human immune system

- The antifungal agents is mostly required in immunocompromised patients such as those receiving cancer chemotherapy, immunosuppressant after organ transplant and HIV patients.



- Fungal kingdom includes yeasts, molds, rusts and mushrooms.
- Fungi could be

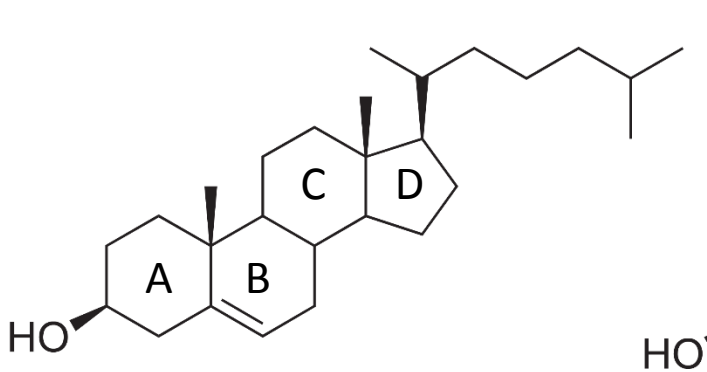
1. Saprophytes, live on dead organic matter.
2. Obligate animal parasite, live only on mammalian hosts such as *Candida albicans* (normal flora of GIT and vagina).
3. Dermatophytes,
   - Live on/in keratin-containing hair, skin and nails of mammals.
   - It attacks the cross-linked structural protein keratin.
   - Called tinea and can infect hair and scalp (tinea capitis), feet (tinea pedis), hand (tinea manuum), nails (tinea ungunium)

Biochemical targets for antifungal agents

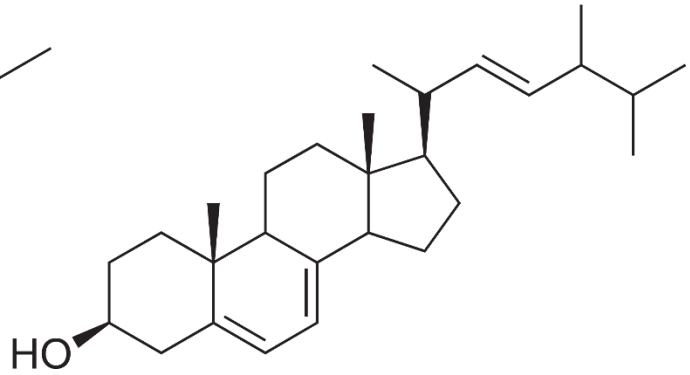
- Antifungal chemotherapy depends on biochemical differences between fungi and mammal cells. Such differences are few since both type of cells are eukarytoics. Example of such differneces

1. Fungal cells have both cell membrane and outer cell wall, whereas mammalian cells only have cell membrane
2. The membranes of fungal and mammalian cells have different types of sterols (i.e. Ergosterol and cholesterol, respectively).

Biochemical targets for antifungal agents (Cont.)

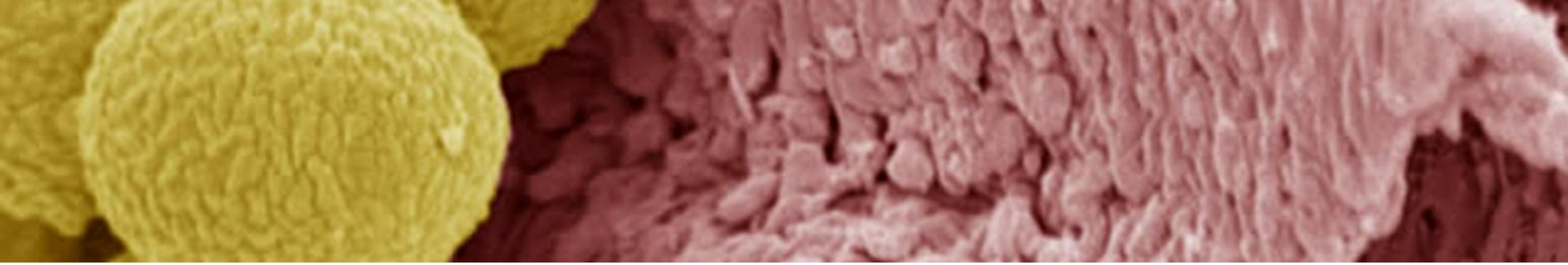


Cholesterol



Ergosterol

- Although the ergosterol and cholesterol are quite similar, the side chains are slightly different, and when three-dimensional models are constructed, the ring system of ergosterol is slightly flatter because of the additional double bonds in the B ring.
- This difference in sterol components provides the biochemical basis of selective toxicity for most of the currently available antifungal drugs.



- The antifungal agents can be divided into the following classes, based on their chemical structure, mechanismof action, and source:

I. **Antibiotics:** Amphotericin B, Nystatin, Griseofulvin

II. **Azoles** (imidazole, triazole derivates)
   - Imidazoles—Clotrimazole, Ketoconazole, Miconazole, Bifonazole, Butoconazole, and Zinoconazole
   - Triazoles—Fluconazole, Itraconzole, Terconazole

III. **Allylamines** Tolnaftate, Naftifine, and Terbinafine

IV. **Fluorinated pyrimidines**: Flucytosine

V. **Chitin synthetase inhibitors**: Nikomycin Z

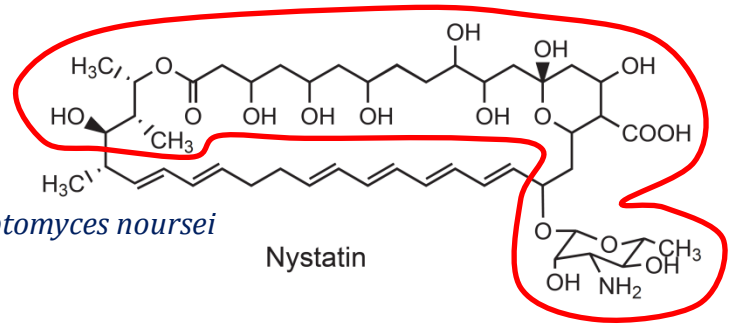
VI. **Peptides/proteins**: Cispentacin

VII. **Fatty and other acids**: propionic acid, undecylenic acid, resorcinol, benzoic acid

VIII. **Miscellaneous**: Ciclopirox,

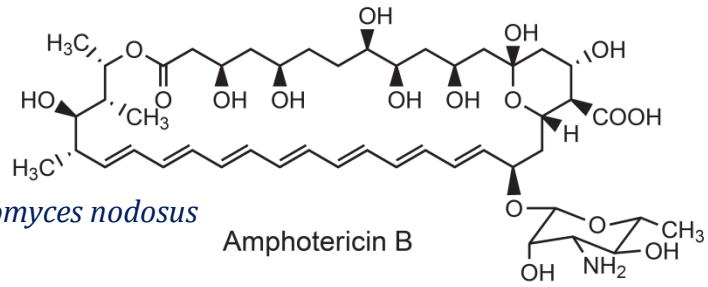
1. Polyene membrane disrupters

- Polyenes are macrocyclic lactones with distinct hydrophilic and lipophilic regions (they are amphoteric amphoteric, forming soluble salts in both basic and acidic environments)
- **Hydrophilic region**: alcohols, carboxylic acid, sugars
- **Lipophilic region**: chromophore of 4 to 7 conjugated double bonds (↑no. of double bonds ➔ ↑ antifungal activity, ↓ toxicity to mammalian cells)



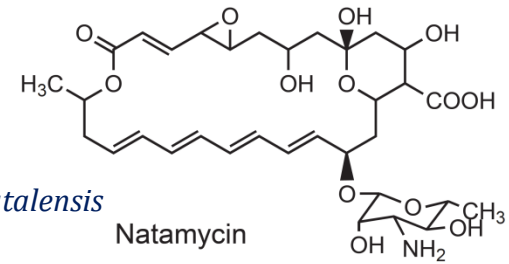
From Streptomyces noursei

Nystatin



From Streptomyces nodosus

Amphotericin B

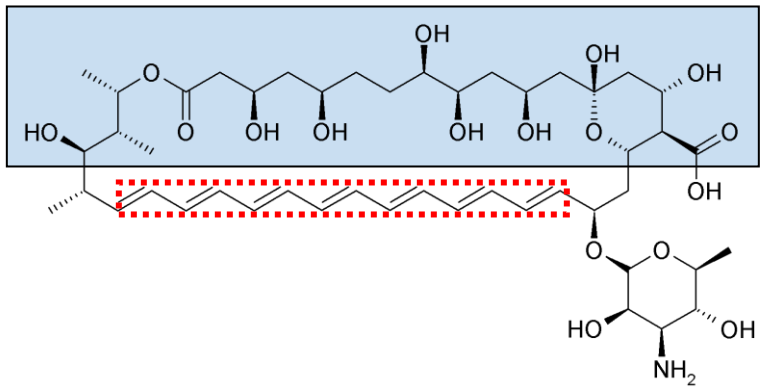


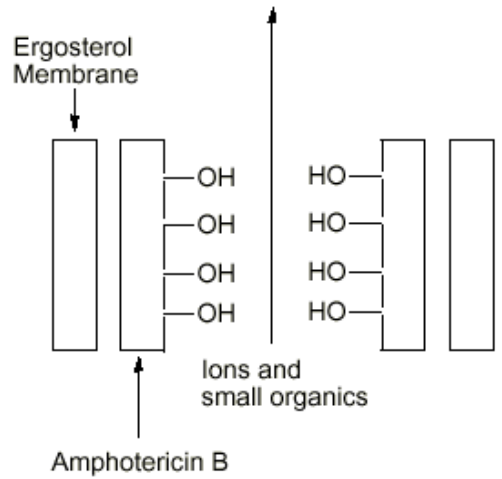
From Streptomyces natalensis

Natamycin

Polyene membrane disrupters (cont.)

Amphotericin





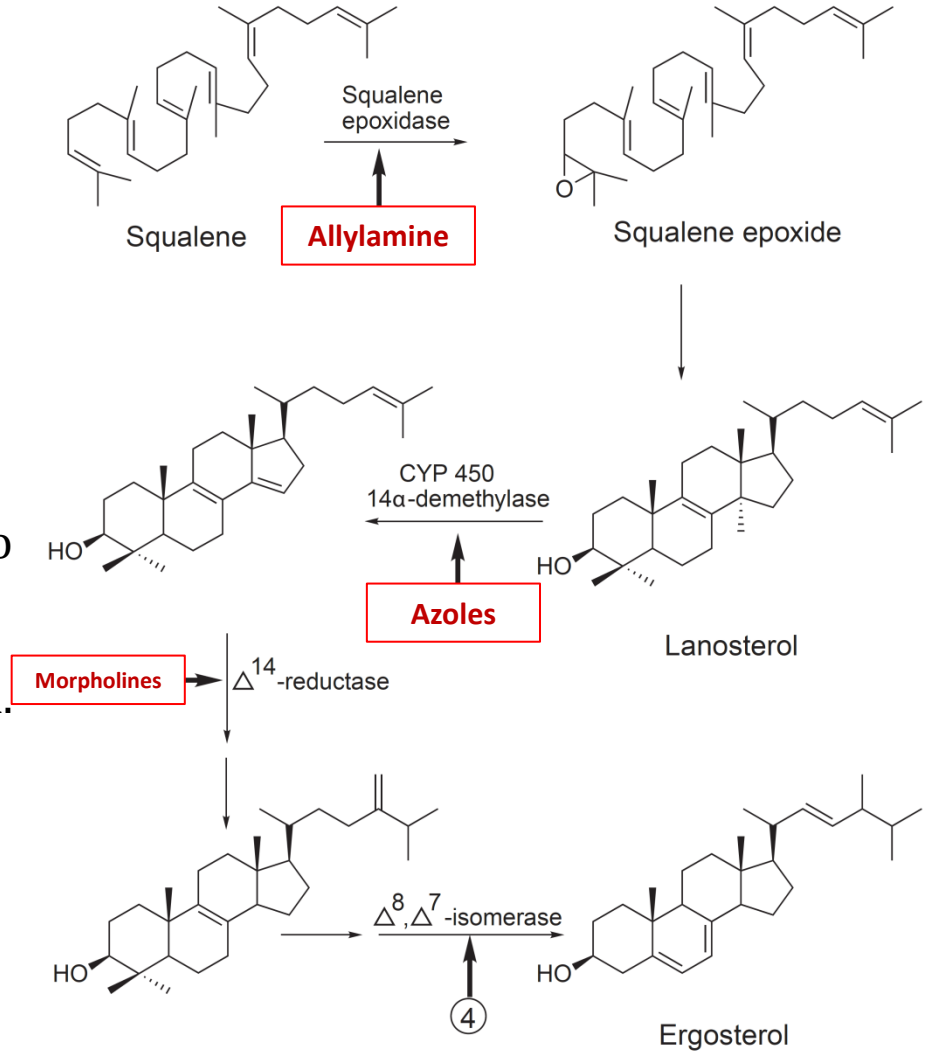
Polyene membrane disrupters (cont.)

- The polyenes have an affinity to sterol-containing membranes, thus being inserted into the membrane, causes leakage and disruption of function.
- The polyenes have higher affinity for ergosterol over cholesterol-containing membrane.
- **NYSTATIN** Nystatin, the first clinically useful polyene antifungal antibiotic, is a conjugated tetraene isolated from cultures of the bacterium *Streptomyces noursei* in 1951. It is too toxic for systematic use and can be used orally to treat GIT infections.
- **AMPHOTERICIN B** Amphotericin B, which as a heptaene has low enough toxicity to mammalian cells to permit intravenous (IV) administration, was discovered in 1956. It can not cross blood-brain barrier. Formulated as water-soluble complex with deoxycholic acid for IV administration.
- **NATAMYCIN**, a tetraene, is available in the United States as a 5% suspension applied topically for the treatment of fungal infections of the eye

2. Ergosterol Biosynthesis Inhibitors

- Fungi biosynthesize ergosterol from squalene precursor (the last nonsteroidal precursor for both erogosterol and cholesterol)

1. Squalene is converted to squalene epoxide by epoxidase
2. Squalene epoxide is cyclized to lanosterol (the first steroid)
3. Lanosterol side chains are modified to ergosterol by reduction, removal of the germinal dimethyl groups (antifungal target), and isomerization.



Ergosterol Biosynthesis Inhibitors (Cont.)

- In inhibition of demethylation step (i.e. accumulation of lanosterol) disrupts the packing of aryl chains of phospholipids, the functioning of certain membrane bound enzyme systems, such as ATPase and enzymes of the electron transport system, and thus, inhibiting the growth of fungi

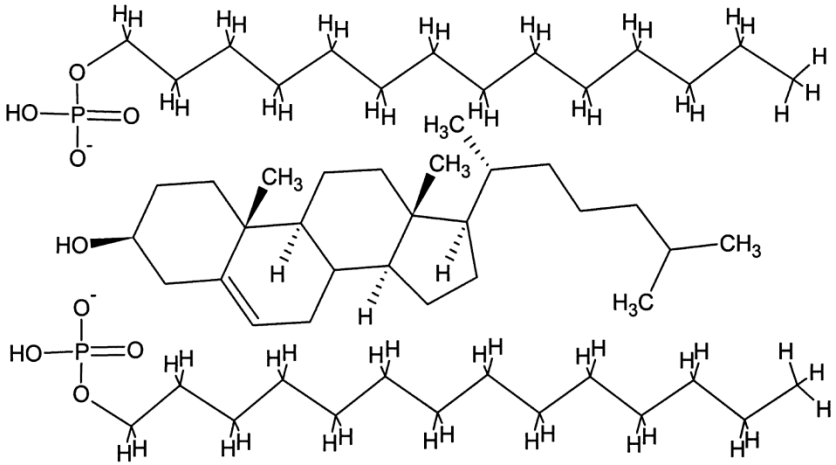


Figure 6.2 ● Ergosterol embedded in a lipid bilayer.

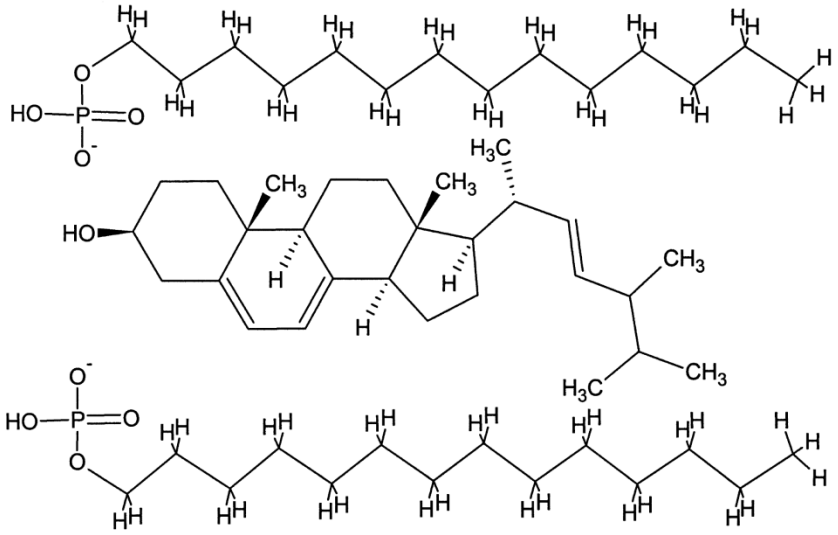


Figure 6.1 ● Cholesterol embedded in a lipid bilayer.

Ergosterol Biosynthesis Inhibitors (Cont.)

- Azole antifungal are the largest class of antimycotics available today.
- Some azoles are used topically, for dermatophytic infections others are used orally to treat systematic infections.
- Unlike amphotericin B, azoles are orally bioavailable and have broader spectrum of activity.
- Azoles have 5-membered aromatic ring containing either two or three nitrogen atoms. The N1 has an aromatic side chain.
- All azoles inhibit 14α-demethylase of ergosterol biosynthesis
- At high in vitro concentrations (micromolar), the azoles are fungicidal; at low in vitro concentrations (nanomolar), they are fungistatic.

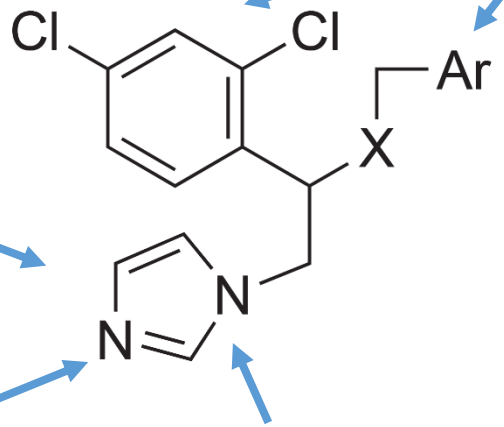
Ergosterol Biosynthesis Inhibitors (Cont.)

- The function of lanosterol 14α-demethylase is to oxidatively remove a methyl group from lanosterol during ergosterol biosynthesis.
- The enzyme is membrane-bound of the class cytochrome P450.
- The enzyme possesses a heme moiety as part of its structure, and the basic electron pairs of the azole rings can occupy a binding site and prevent the enzyme from turning over
- The enzyme is also present in mammalian biosynthesis of cholesterol, and the azoles are known to inhibit cholesterol biosynthesis also (e.g. biosynthesis of adernocorticoids)
- The mammalian copy of the enzyme is much sensitive and binds azoles with lower affinity than fungal copy (which explains the selective fungal toxicity).
- The 1,2,4-triazoles appear to cause a lower incidence of endocrine effects and hepatotoxicity than the corresponding imidazoles, possibly because of even lower affinity to mammalian copy of the enzyme.

SAR for Azoles

large nonpolar portion of the molecule mimics the nonpolar steroidal part of the substrate for lanosterol

imidazole ring can be replaced with a bioisosteric 1,2,4-triazole ring (the last binds with lower affinity to mammalian copy of demethylase → less endocrine and hepatotoxicity)



The amidine nitrogen atom (N-3 in the imidazoles, N-4 in the triazoles) bind to the heme iron of enzyme-bound cytochrome P450 and **should not be substituted**

A weakly basic imidazole or 1,2,4-triazole ring (pK_a of 6.5–6.8) connected through N1 is important

- The most potent antifungal azoles possess two or three aromatic rings, at least one of which is halogen substituted
- Only 2, and/or 2,4 substitution yields effective azole compounds (Substitution at other positions of the ring yields inactive compounds)
- The most potent halogen is fluorine, although other groups like sulfonic acids do the same

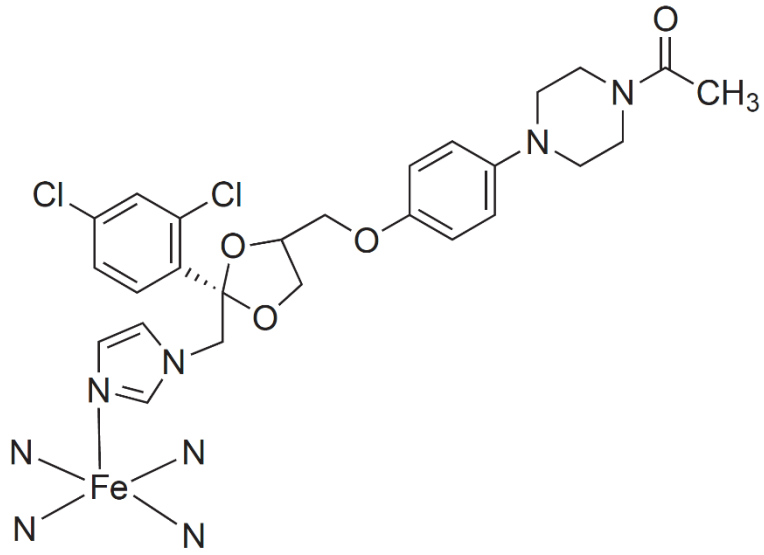
Azoles (cont.)

- The early azole antifungal drugs were all either extensively and rapidly degraded by first-pass metabolism or too toxic for systemic use.
- Drugs with reduced or slow first-pass metabolism are used systematically such as ketoconazole, fluconazole, itraconazole, voriconazole, and posaconazole
- Drugs available as creams and ointments for dermatophytic and vaginal infections include clotrimazole, tioconazole, terconazole, butoconazole, econazole, oxiconazole, sulconazole, miconazole, and ketoconazole

Azoles (cont.)

1- Ketoconazole

- An imidazole antifungal, was the first orally active antifungal azole
- Absorption depends on low stomach pH (not used with antacids)
- Extensively metabolized by microsomal enzymes to inactive metabolites. Therefore, it ketoconazole is a powerful inhibitor of human CYP3A4 leading to interaction with metabolism of other drugs especially those with narrow therapeutic index.



14α-Demethylase heme

Mechanism of azole/CYP450 binding. The basic nitrogen of azole antifungal agents forms a bond to the heme iron of CYP450 enzymes, preventing the enzyme from oxidizing its normal substrates. Ketoconazole is representative of the azole antifungals.

Azoles (cont.)

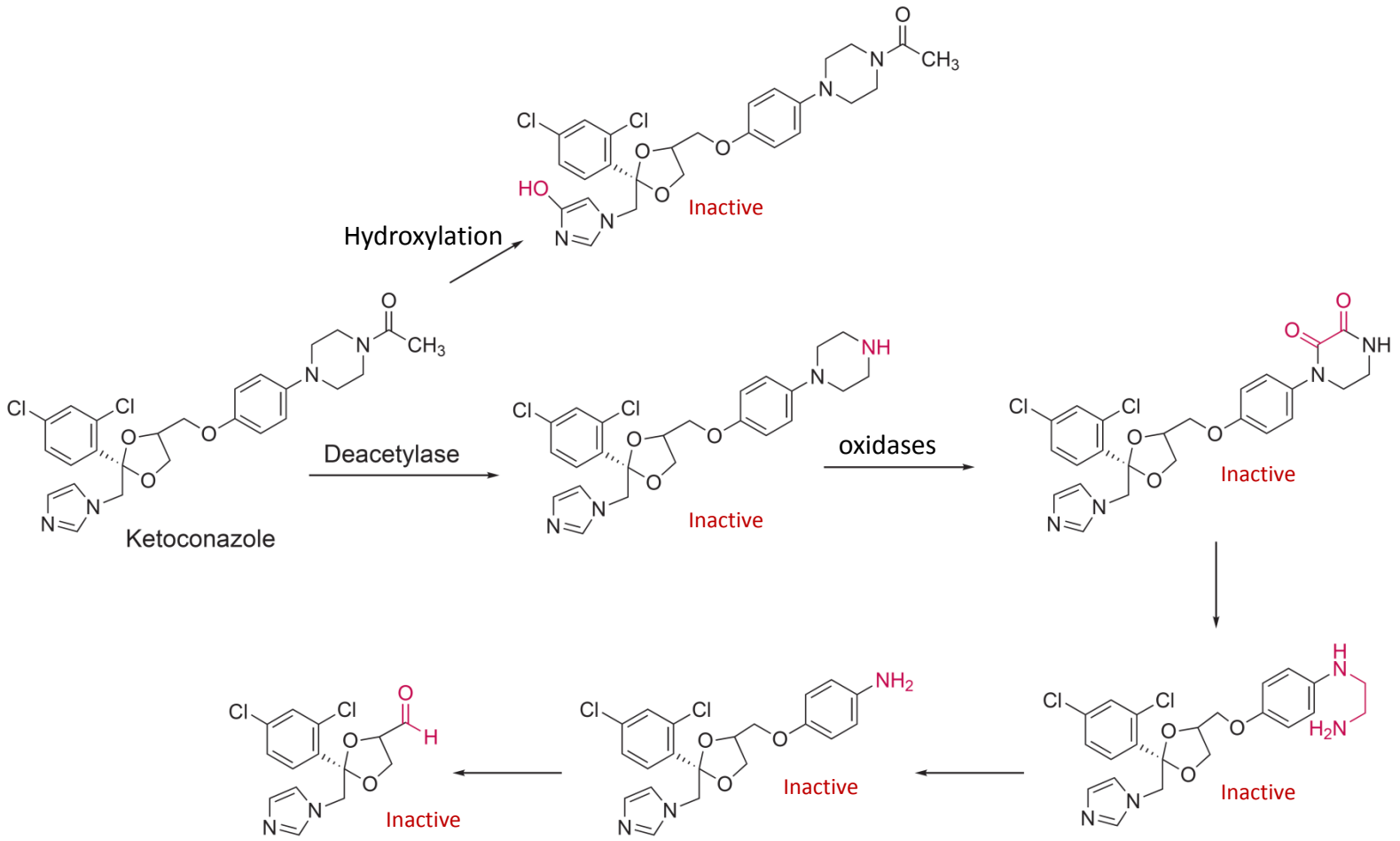
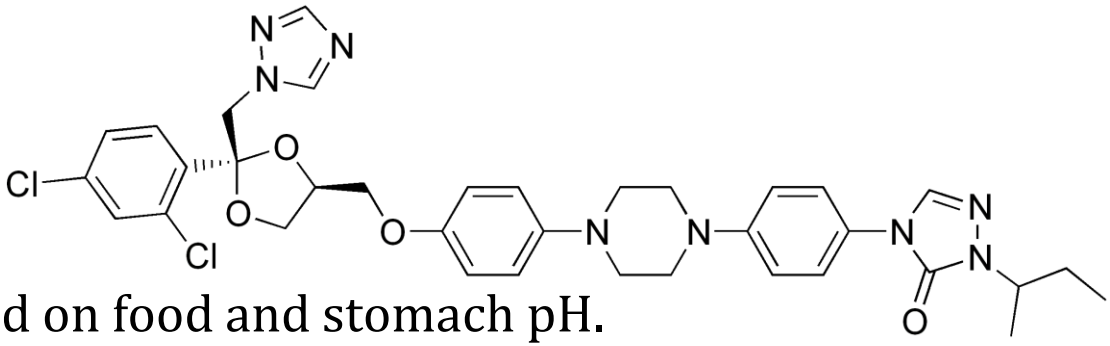


FIGURE 35.5 Extensive metabolism of ketoconazole involving hydrolysis of the *N*-acetyl by a deacetylase. The oxidation reactions are catalyzed by CYP3A4 and flavin-linked mixed-function oxidase. All metabolites are inactive.

Azoles (cont.)

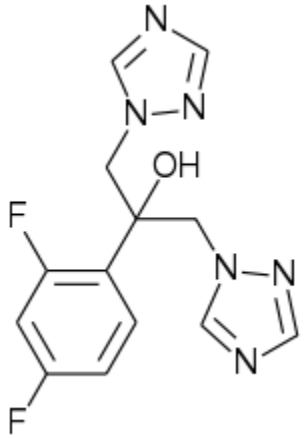
2- Itraconazole:



- Triazole derivative.
- Oral bioavailability depend on food and stomach pH.
- highly interfere with liver enzymes (serious drug drug interactions).

3- Fluconazole:

- Equal bioavailability, oral and I.V. (Not affected by stomach pH)
- Could cross BBB (Why?).
- Weak inhibitor to some liver enzymes.



Azoles (cont.)

- All azoels are nonpolar lipophilic, thus their free bases are insoluble in water but soluble in organic solvents such as ethanol
- Fluconazole, which possesses two polar triazole moieties, is an exception, in that it is sufficiently water soluble to be injected intravenously as a solution of the free base.

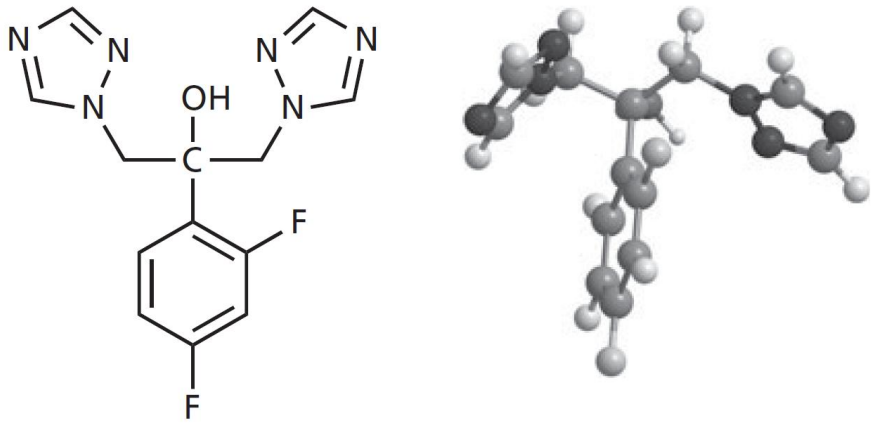
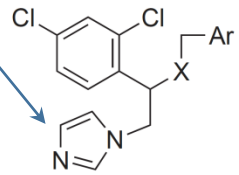
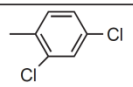
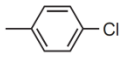
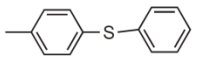
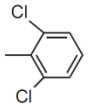
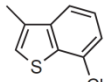


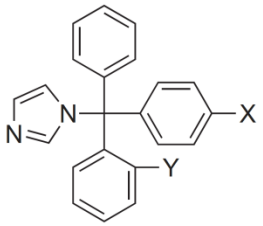
FIGURE 12.2 Fluconazole.

Examples of Azoles

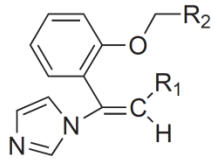
Imidazole ring

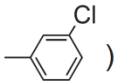


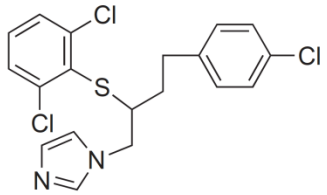
Generic name	X	Ar
Miconazole	O	
Econazole	O	
Sulconazole	S	Same
Fenticonazole	O	
Isoconazole	O	
Sertaconazole	O	
Tioconazole	O	



Clotrimazole (X = H, Y = Cl)
Flutrimazole (X = Y = F)

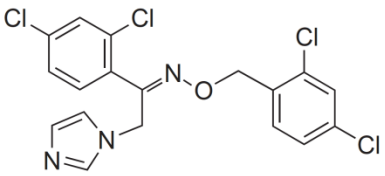


Croconazole (R_1 = H, R_2 = )

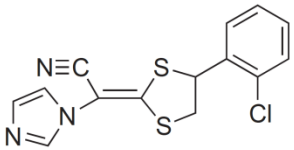


Butoconazole

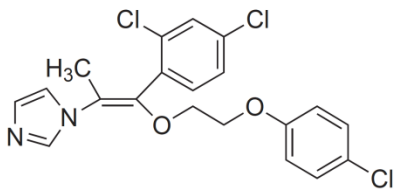
Neticonazole (R_1 = SCH_3, R_2 = n-C_4H_9)



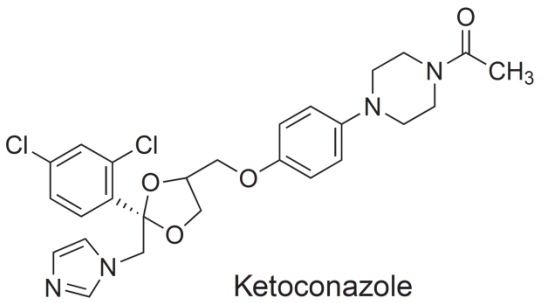
Oxiconazole



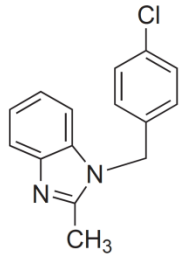
Lanoconazole



Omoconazole



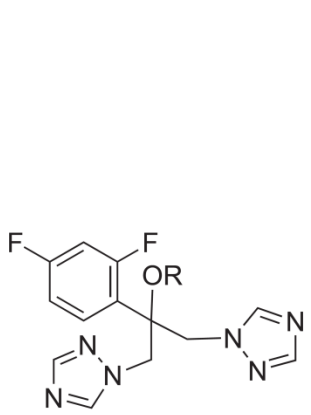
Ketoconazole



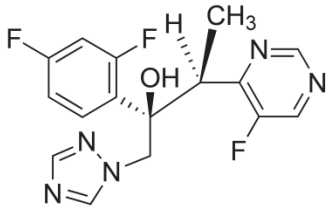
Chlormidazole

FIGURE 35.3 Imidazole antifungal agents.

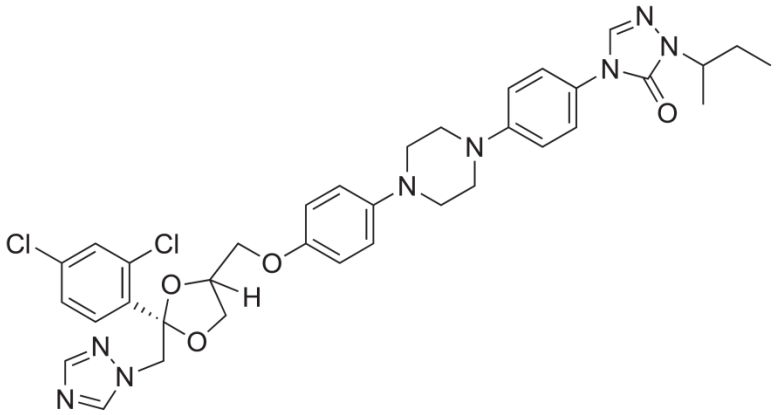
Examples of Triazoles



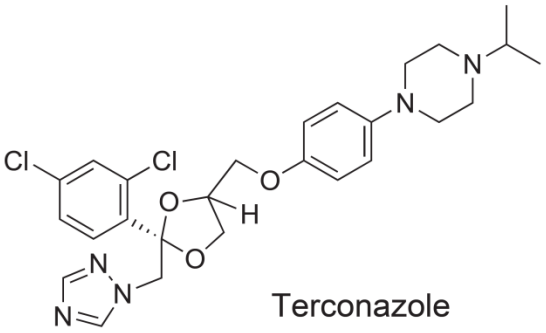
Fluconazole (R = H)
Fosfluconazole (R = —P(=O)(OH)OH)



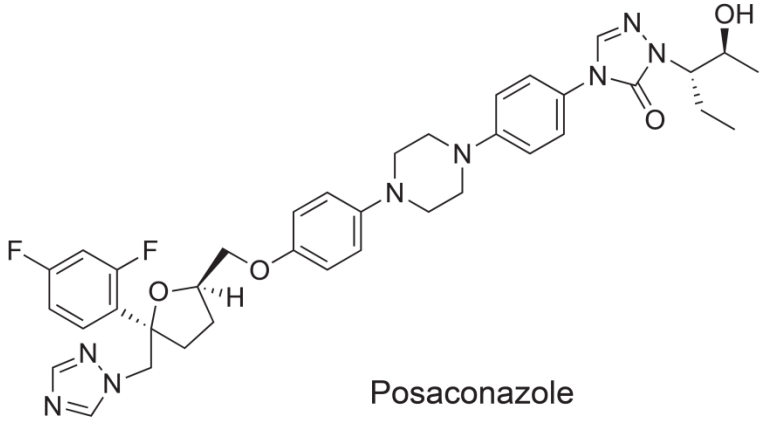
Voriconazole



Itraconazole



Terconazole



Posaconazole

FIGURE 35.6 Triazole antifungal agents.

Resistance to azoles

- Resistance to imidazoles was observed especially in *Candida albicans* due to

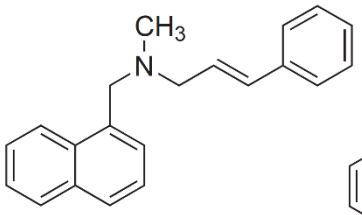
1. Mutation in the 14α-demethylase: thus azoles are no more able to bind heme while binding to lanosterol is preserved.
2. Increasing efflux by ATP-binding cassette (ABC-1, which normally transports cholesterols)
3. Increase production of 14α-demethylase

3. Squalene epoxidase inhibitors (Allylamines)

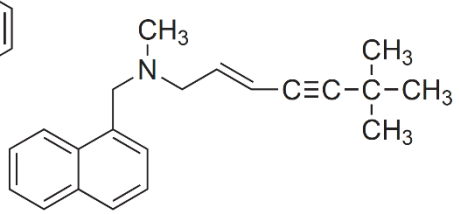
- Allylamines have narrower spectrum (compared to azoles) and only effective against dermatophytes.
- Used for infections of skin and nails
- Inhibit the squalene epoxidase thus
  - Decrease total sterol contant of the fungal cell membrane
  - Increase in squalene deposition which is toxic in high amount
- Allylamines bind with lower affinity to mammalian squalene oxidases (Terbinafi ne has a *K*i of 0.03 mmol/L versus squalene epoxidase from *Candida albicans* but a *K*i of only 77 mmol/L versus the same enzyme from rat liver—a 2,500-fold difference)

Allylamines (Cont.)

- Extensive first-pass effect
- Not used orally
- Used mainly for Tinea of skin



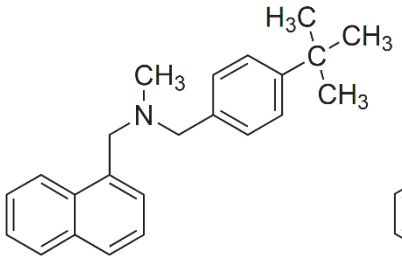
Naftifine



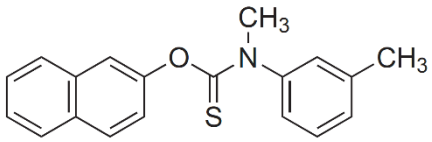
Terbinafine

- Extensive first-pass effect
- Used topically and orally for dermatophytic infections
- Lipophilic → can distribute to nails

- Only available in topical prepartions
- Butenafine have wider spectrum of activity than tolnaftate

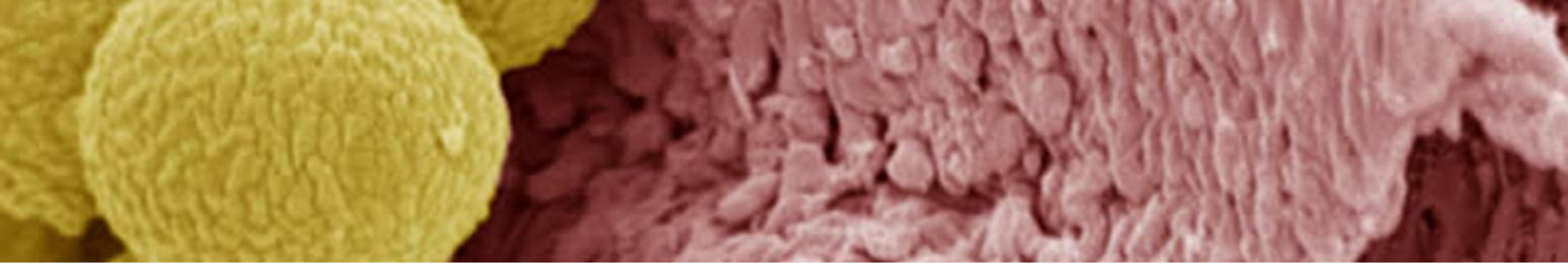


Butenafine



Tolnaftate

FIGURE 35.8 The squalene epoxidase inhibitors, allylamines. Naftifine was the first drug shown to act by inhibition of squalene epoxidase as was the much older thiocarbamate, tolnaftate.



- Griseofulvin is a fungi-static drug that causes disruption of the mitotic spindle by interacting with polymerized microtubules