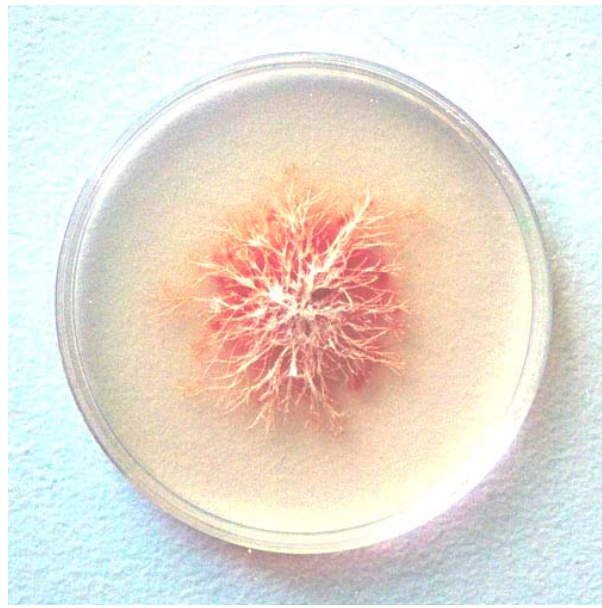
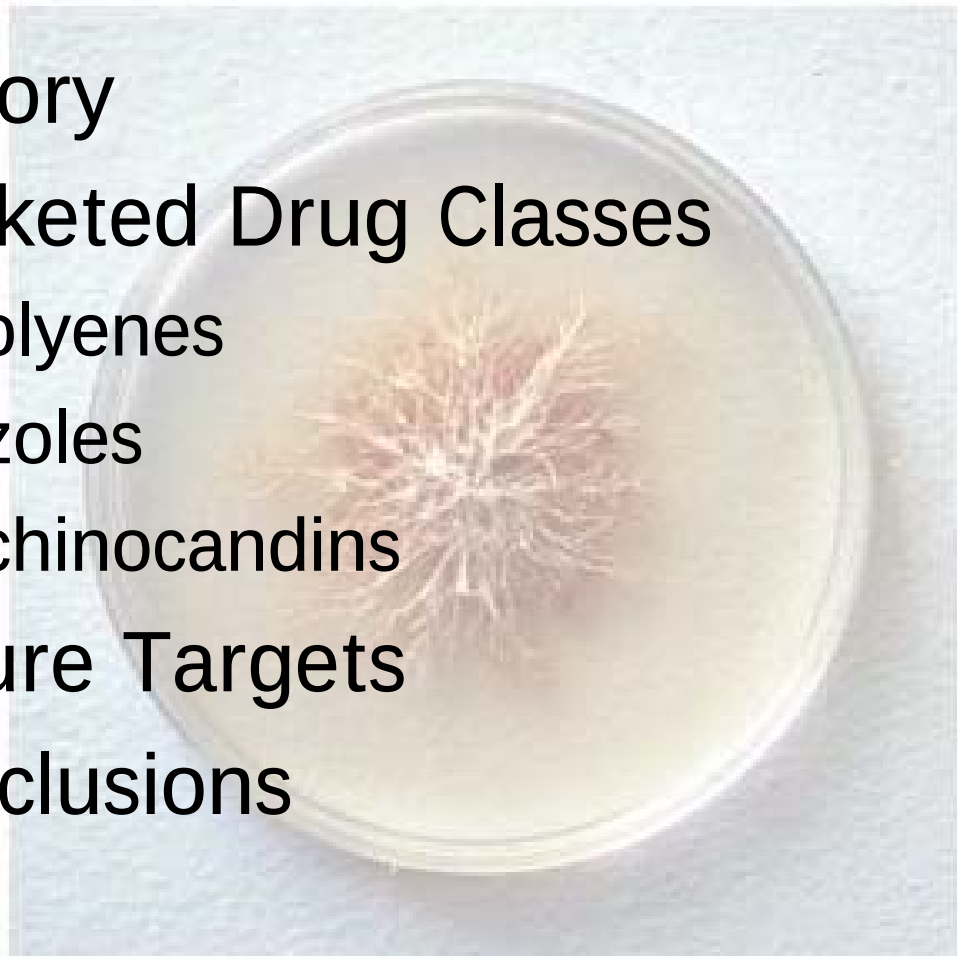
Recent Advances in Antifungal Drug Development

Jennifer O'Neill

February 2, 2006



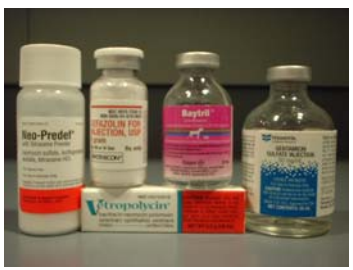
Outline

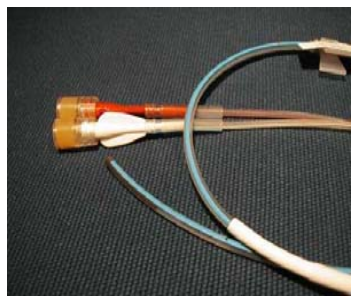


- History
- Marketed Drug Classes
  - Polyenes
  - Azoles
  - Echinocandins
- Future Targets
- Conclusions

Dramatic Increase

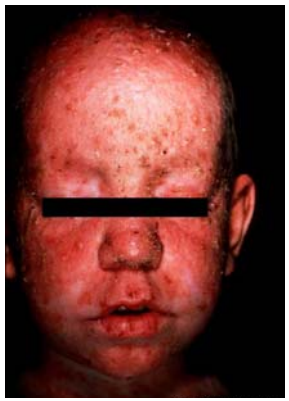
- 300% as many hospital-acquired fungal infections
  - Increase in immunocompromised population (HIV/AIDS)
  - Changes in medical practice
    - Immunosuppressive drugs
    - Harsh chemotherapy
    - Indwelling catheters
    - Indiscriminate use of broad spectrum antibiotics



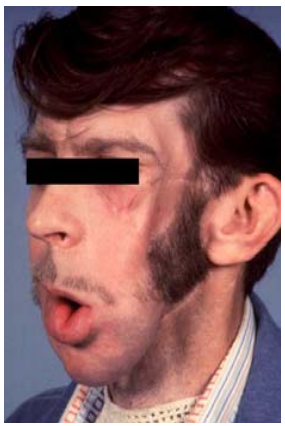


Current Treatment Options in Infectious Diseases **2003**, *5*, 489.
Images from web.princeton.edu and www.sai1.net

Types of Fungal Infections



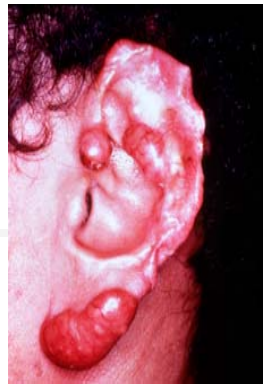
- Candidiasis – *Candida albicans*
  - Impaired immunity, receiving broad-spectrum antibiotic treatment
  - 80% of hospital-acquired infections
  - Mortality rate ~ 40%



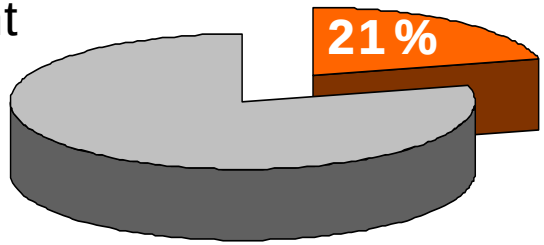
- Aspergillosis – *Aspergillus* spp.
  - Impaired immunity, corticosteroid recipients
  - 1/3 infected – never received antifungal therapy
  - Mortality rate ~ 80%

de Pauw, B. E.; Meuier F. *Chemotherapy* **1999**, *45*, 1.
Images from DoctorFungus Corporation

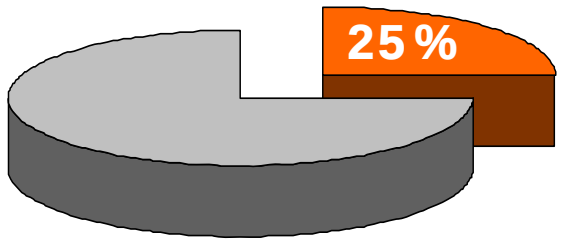
Impact of Infections



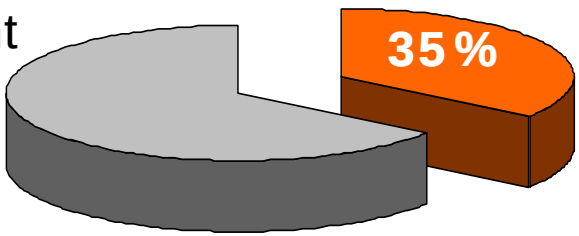
Heart transplant patients die of invasive aspergillosis



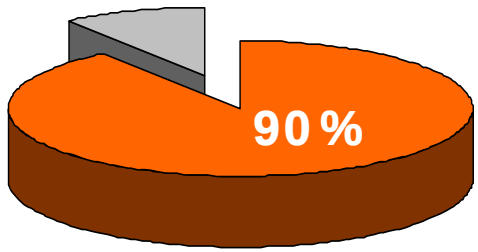
Infection-related deaths in leukemia patients



Lung transplant patients die of invasive aspergillosis

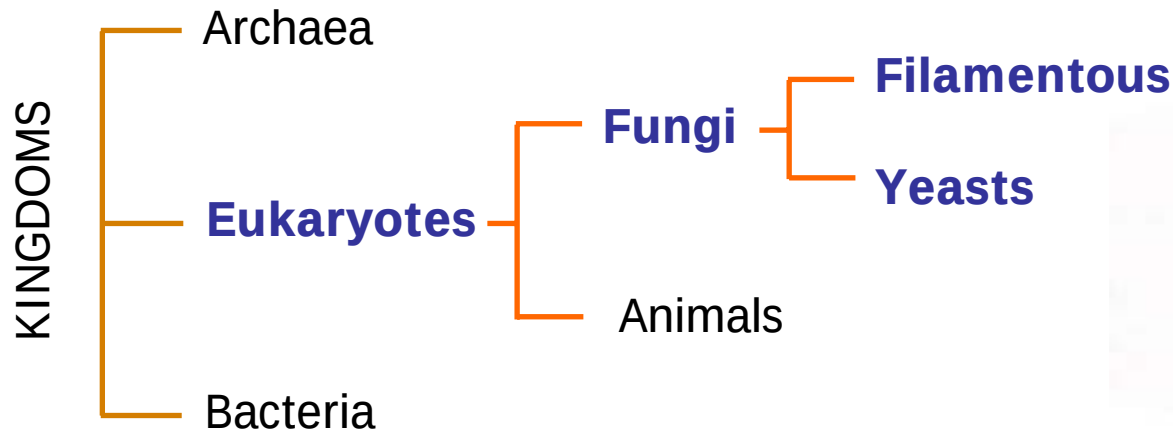


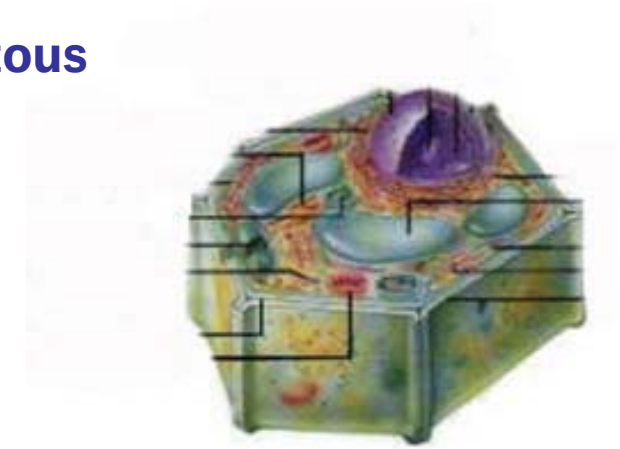
HIV/AIDS patients will contract fungal infections



de Pauw, B. E.; Meunier F. *Chemotherapy* **1999**, *45*, 1.
Image from DoctorFungus Corporation

Fungi Challenging to Target





- Cellular similarities
  - Complicates target identification
- Diversity of structure
- Diversity of metabolic targets

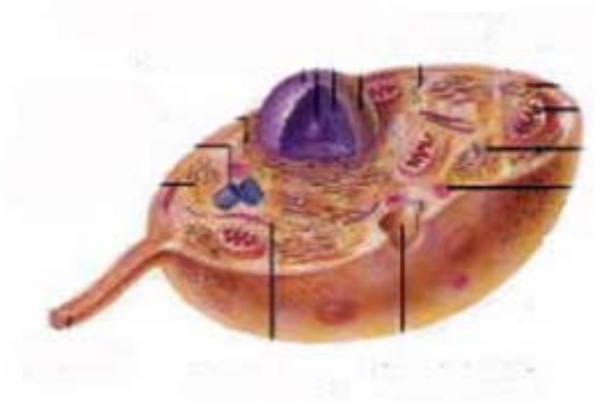


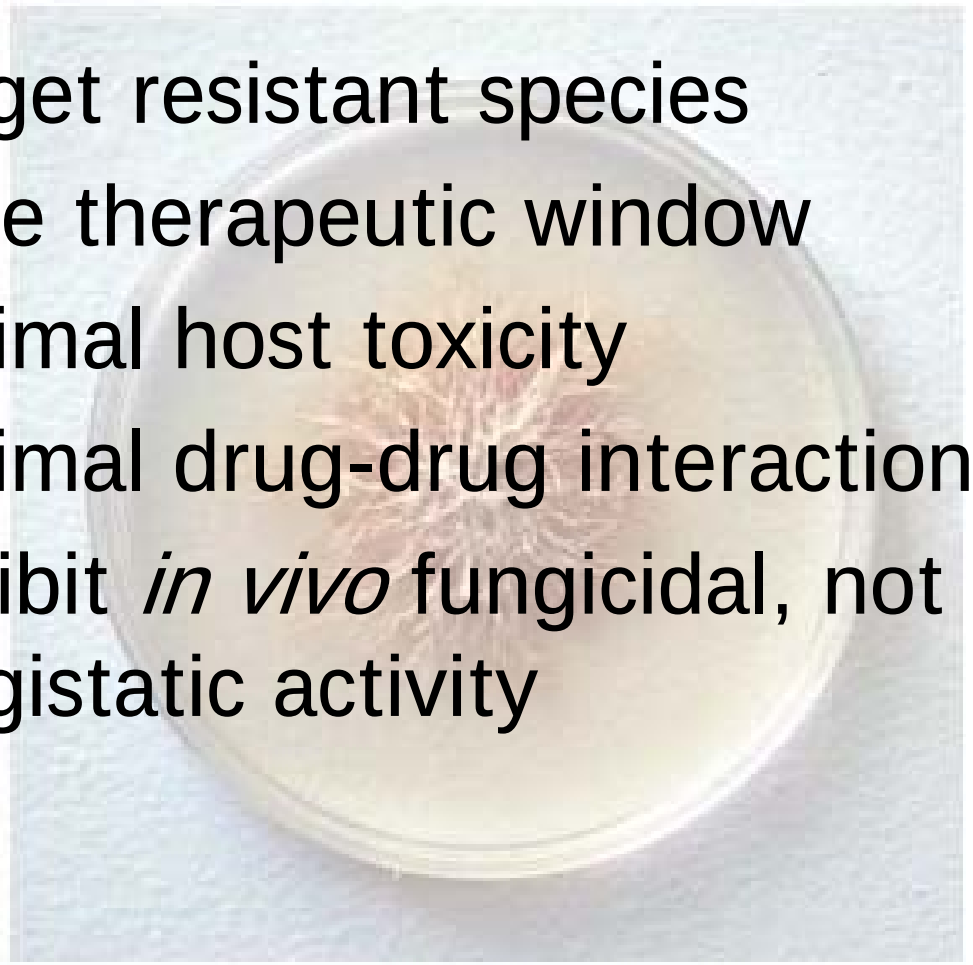
Image from kvhs.nbed.nb.ca

Too Few Antifungals

- Genetic tools unavailable
- Down-played for many decades
  - Far fewer infections (until 1980s)
- Inhibitory cost

- 200 patents from 1998–2000
  - 10–12 years to clinic

Necessary Characteristics

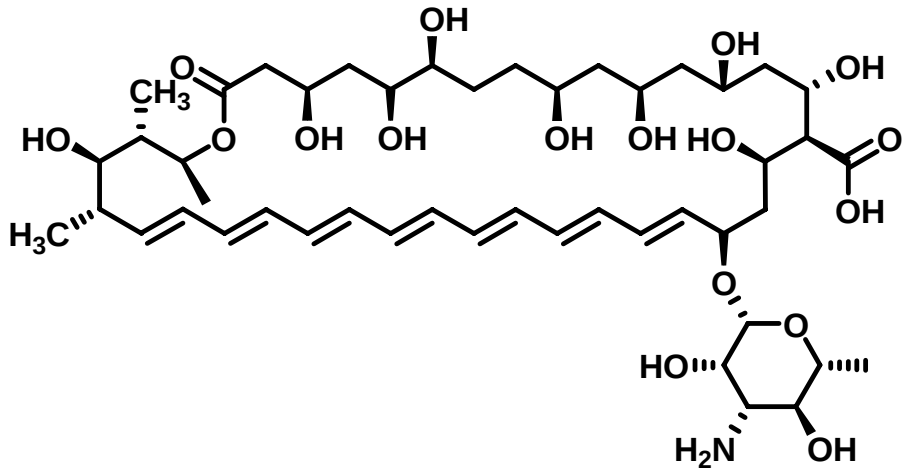


- Target resistant species
- Wide therapeutic window
- Minimal host toxicity
- Minimal drug-drug interactions
- Exhibit *in vivo* fungicidal, not fungistatic activity

Current Treatment Options in Infectious Diseases **2003**, *5*, 489.

Antifungal Classes

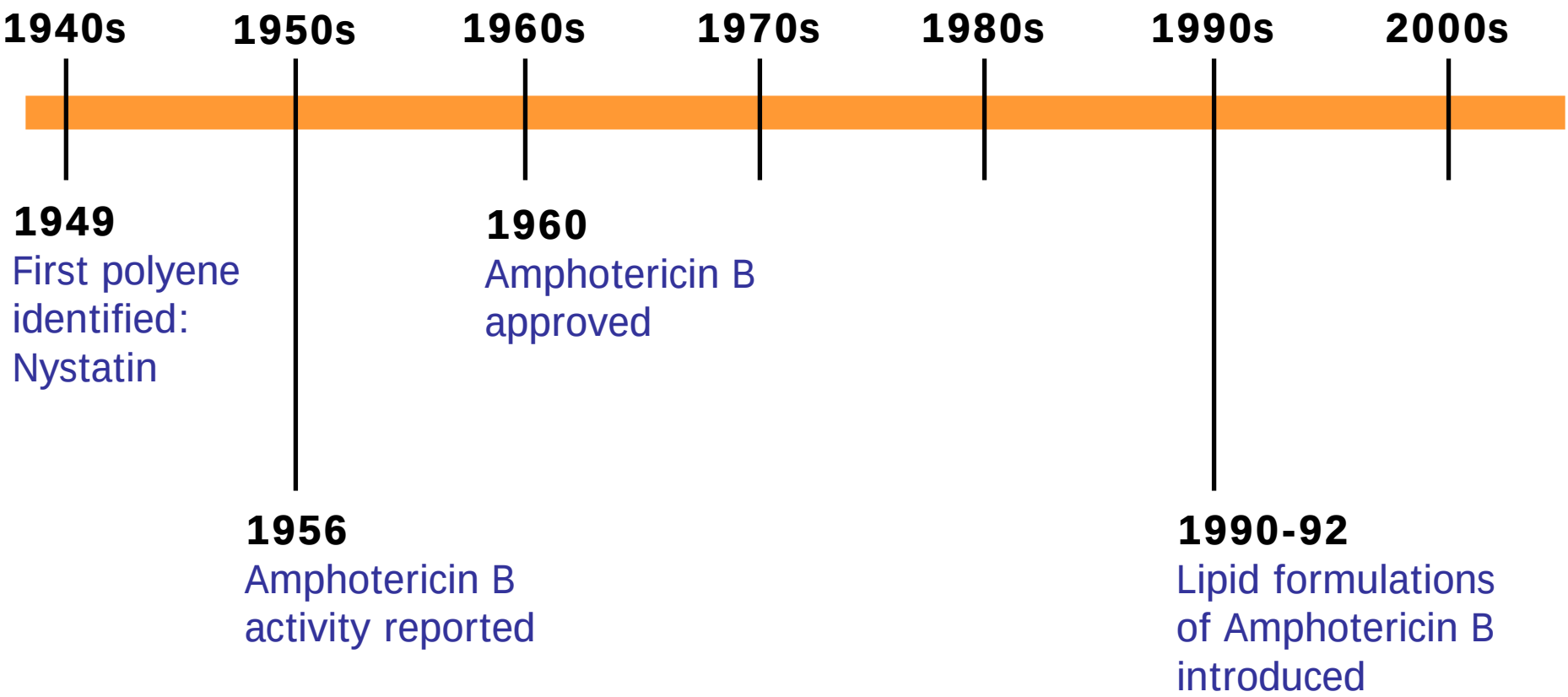
- Polyenes — bind ergosterol
- Azoles — inhibit ergosterol synthesis
- Echinocandins — inhibit glucan synthase
- Allylamines — inhibit squalene epoxidase
- Nikkomycins — chitin synthesis inhibitors
- Sodarins — inhibit protein synthesis
- *N*-Myristoyl transferase inhibitors
- Sphingolipid synthesis inhibitors



Polyenes

Binding ergosterol

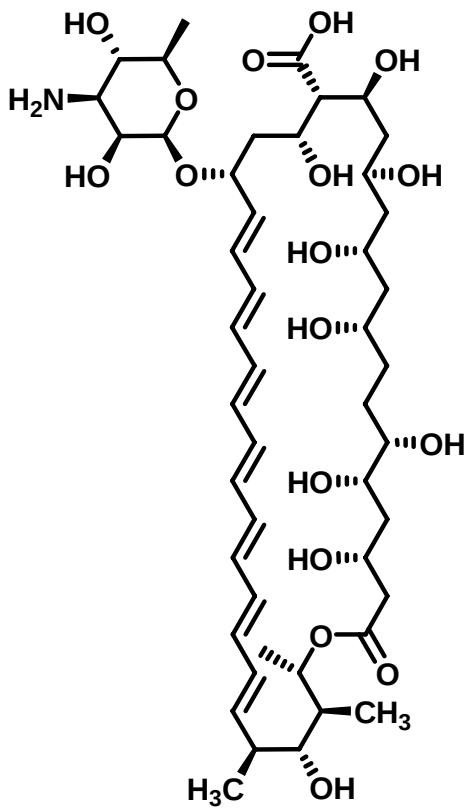
Key Events in Polyene History



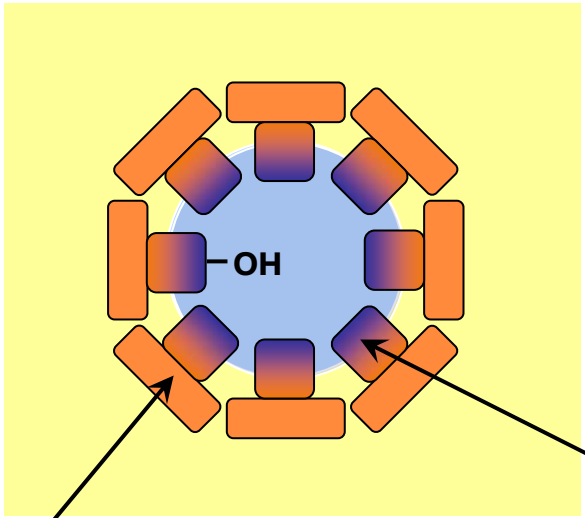
Sheehan, D. J. *et al*. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(1), 40

Amphotericin B

- Isolated from bacteria in 1956
  - *Streptomyces noursei*
- The gold standard
  - Most effective antifungal for over three decades
  - Fungicidal
  - Limited to fungi that contain sterols

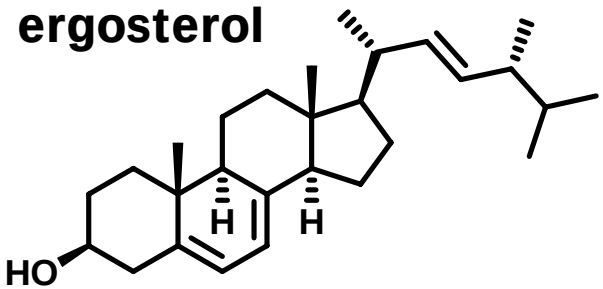


Mechanism of Action

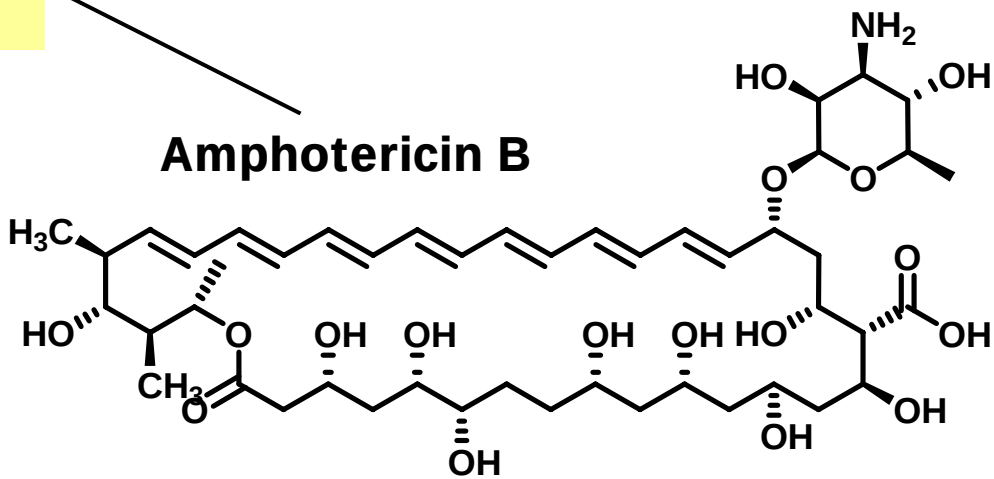


- Amphotericin B binds to ergosterol in cell membrane
- Alters permeability of membrane

ergosterol

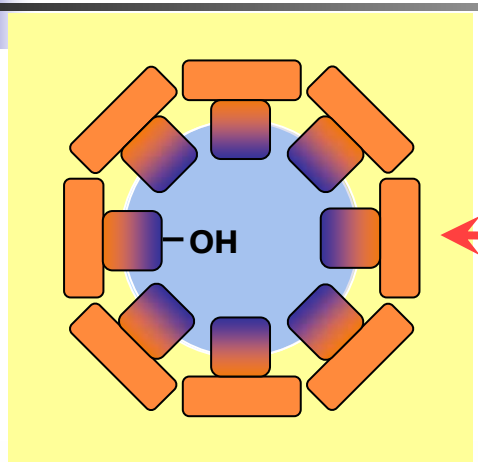


Amphotericin B



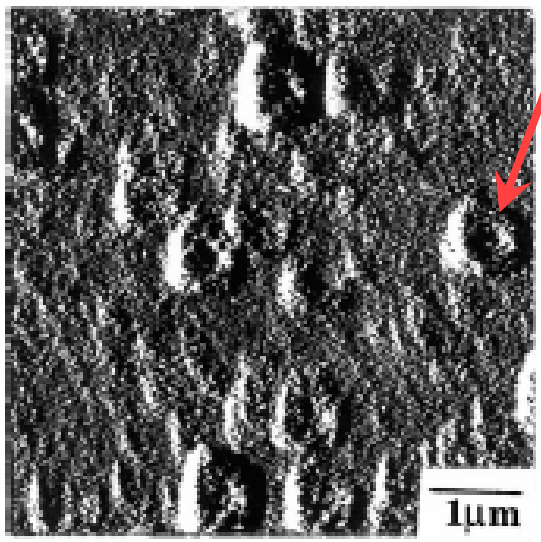
Ghannoum, M. A.;Rice L. B. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(4), 501.
Milhaud, J. *et al*. *Biochim. Biophys. Acta* **2002**, *1558*, 95.

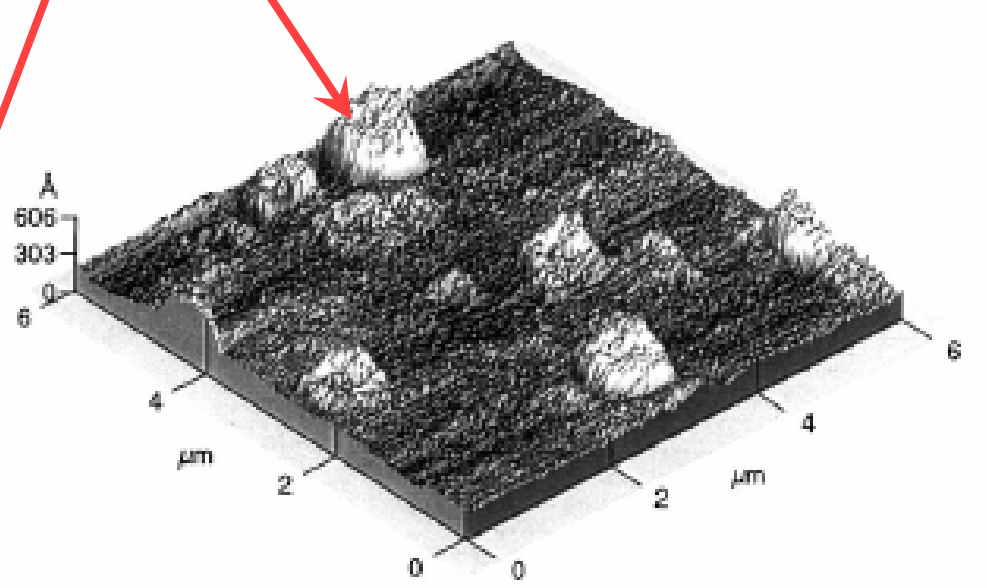
Mechanism of Action



aggregates

Aqueous pores cause leakage of vital cytoplasmic components



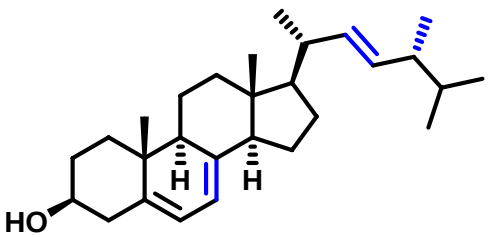


Ghannoum, M. A.; Rice L. B. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(4), 501.
Milhaud, J. *et al*. *Biochim. Biophys. Acta* **2002**, *1558*, 95.

Limitations of Amphotericin B

- Drug of last resort – highly toxic

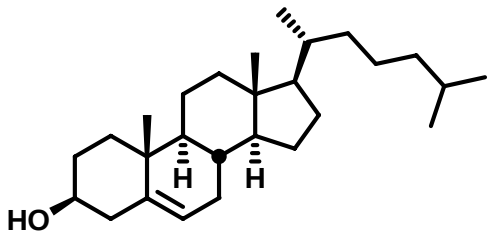
FUNGAL



Ergosterol

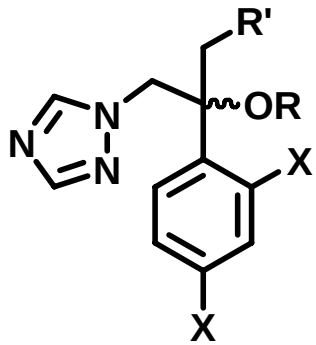
vs.

MAMMALIAN



Cholesterol

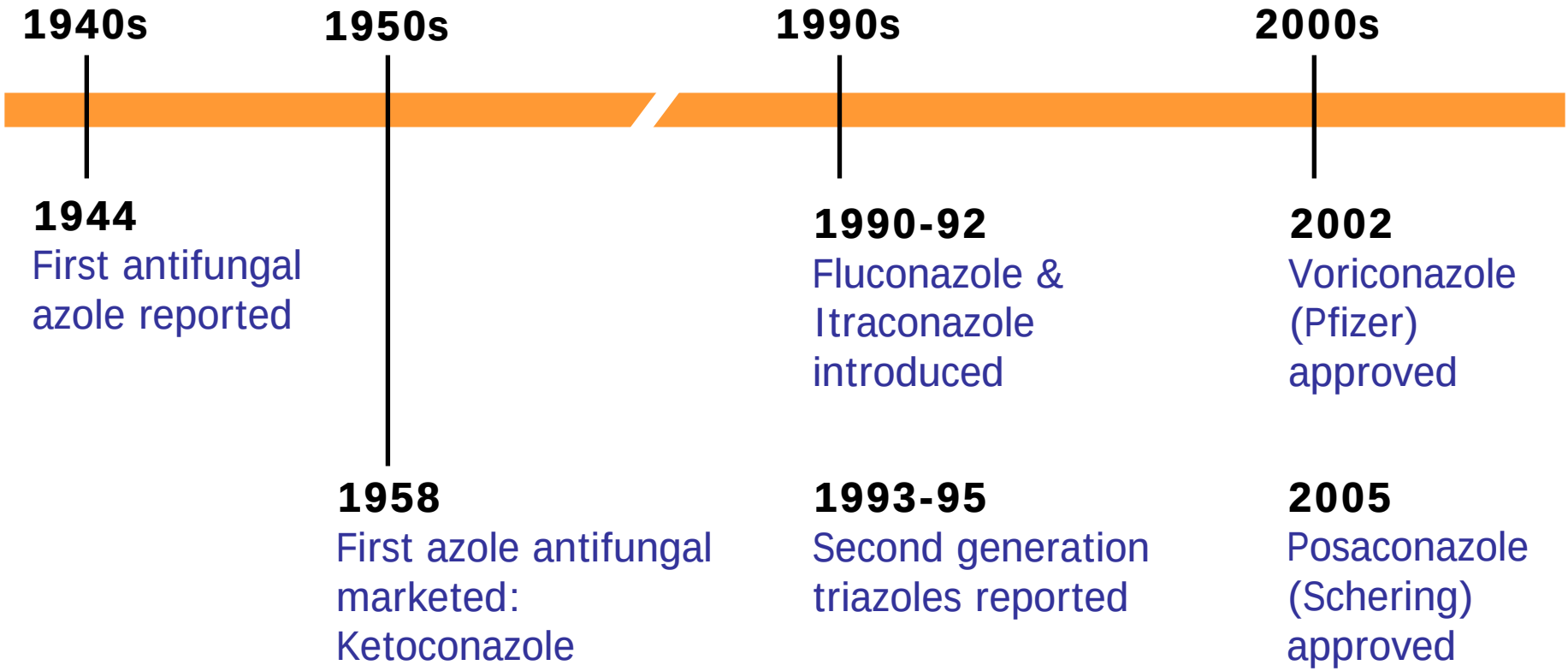
- Resistance has been reported
  - Fungi alter membrane composition



Azoles

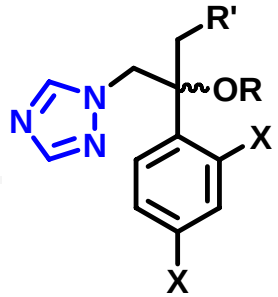
Blocking ergosterol synthesis

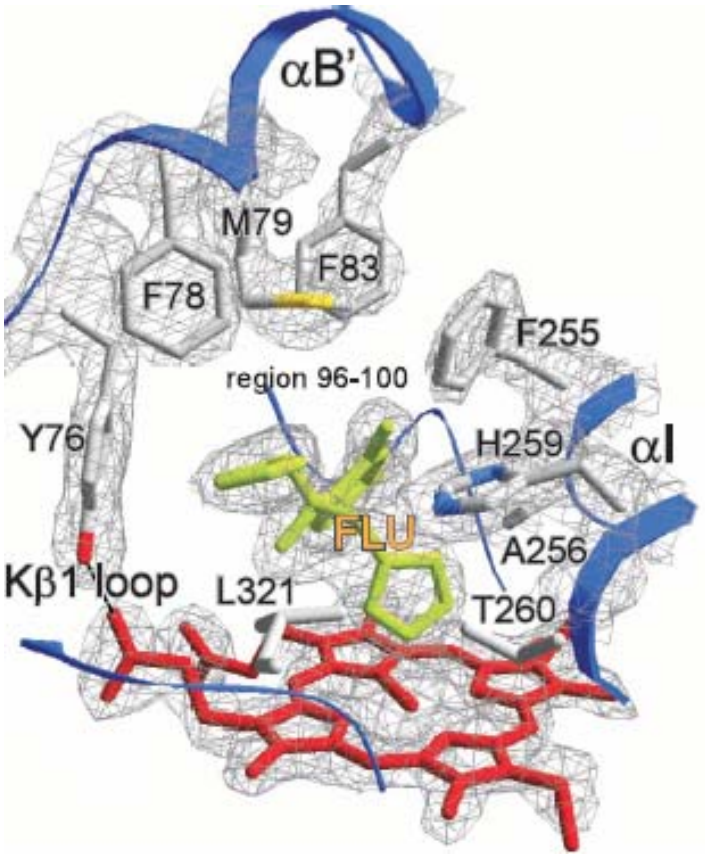
Key Events in Azole History

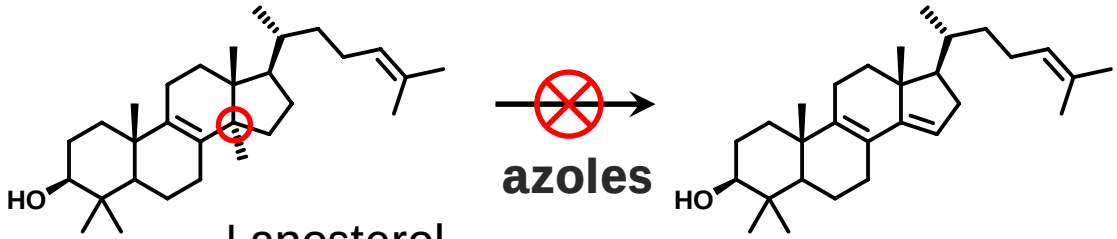


Sheehan, D. J. *et al*. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(1), 40

Mechanism of Action







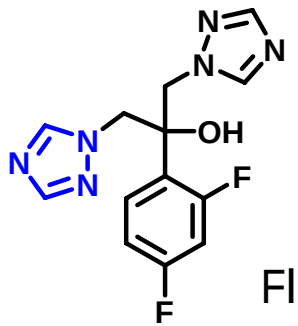
Lanosterol

- Inhibits cytochrome P450 14α-demethylase
- Fungistatic, not fungicidal

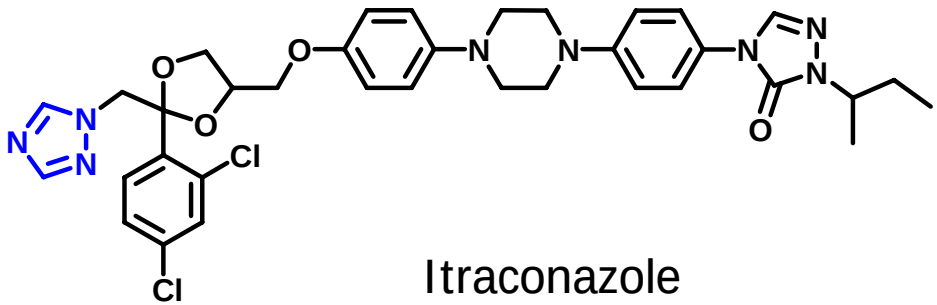
Ghannoum, M. A.; Rice L. B. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(4), 501.
Image from Podust, L. M. *et al*. *PNAS* **2001**, *98*(6), 3068.

1st Generation Triazoles

- Major impact on management of fungal infections in 1990s
- Broad spectrum of activity
  - Yeasts and filamentous fungi
- 1999: >15 marketed azoles worldwide

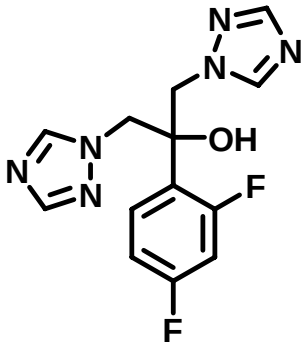


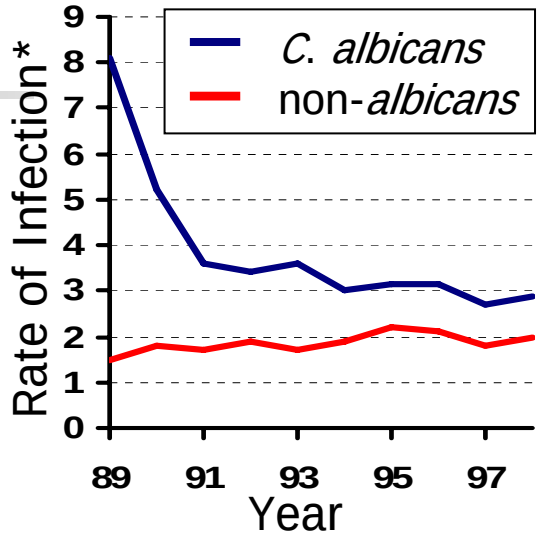
Fluconazole



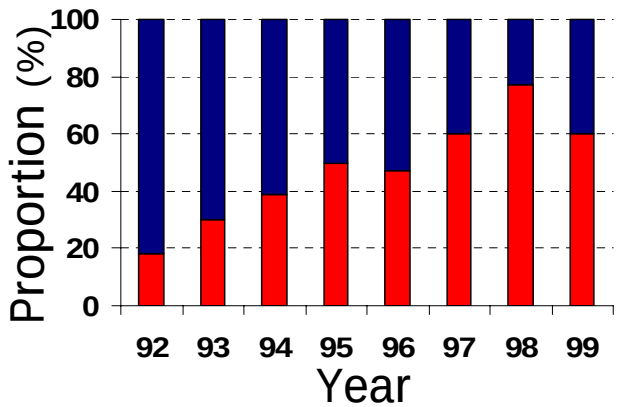
Itraconazole

Fluconazole





- High safety profile – extensive use
- Not active against *Aspergillus* spp.
- Increasing reports of antifungal resistance

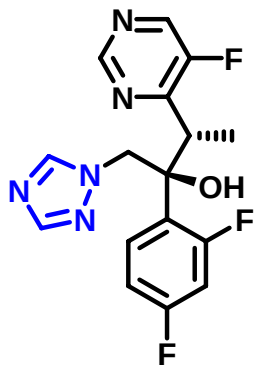


*blood stream infections/
10,000 central venous catheter days

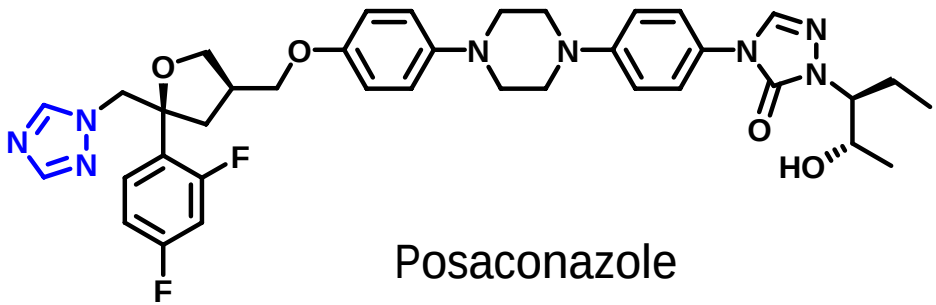
Ghannoum, M. A.; Rice L. B. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(4), 501.
Trick, W. E. *et al*. *Clin. Infect. Dis.* **2002**, *35*, 627. Hope, W. *et al*. *J*. *Hosp. Infect.* **2002**, *50*, 56.

2nd Generation Triazoles

- Enhanced potency (10–500x) over 1st generation
- Broad-spectrum activity: yeasts, molds, *Aspergillus*
- Excellent central nervous system penetration
- Greatly reduced toxicity



Voriconazole

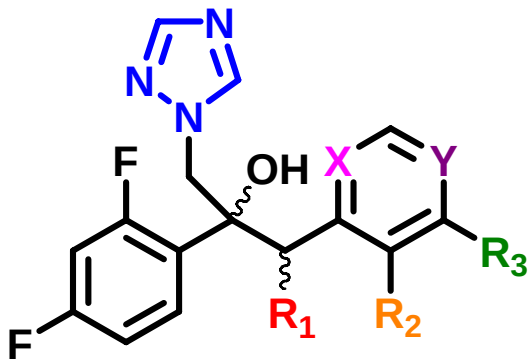


Posaconazole

Koltin Y.; Hitchcock C.A. *Curr*. *Opin*. *Chem*. *Biol*. **1997**, *1*(2), 176.
Groll A. H.; Walsh, T. J. *Swiss Med. Wkly.* **2002**, *132*, 303.

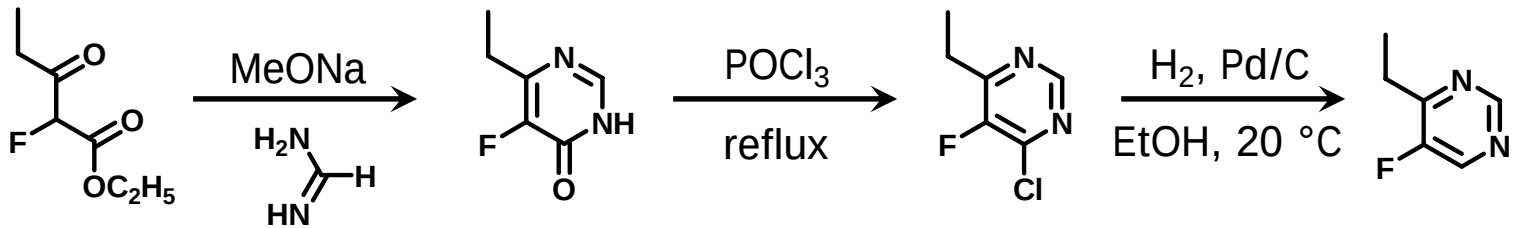
Derivatives of Fluconazole

Wanted to increase spectrum of activity to include *Aspergillus* spp.



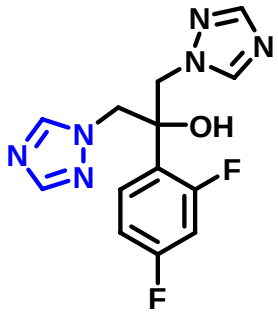
R_1 = H, Me
R_2 = H, F, Cl
R_3 = H, Cl
X =N, CH
Y = N, CH

Synthesis of fluoropyrimidine

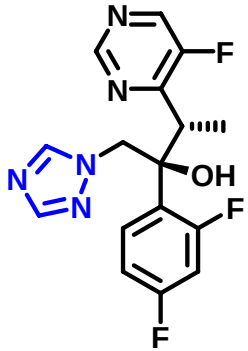


Dickinson R. F. *et al*. *Bioorg*. *Med*. *Chem*. *Lett*. **1996**, *6*(16), 2031.

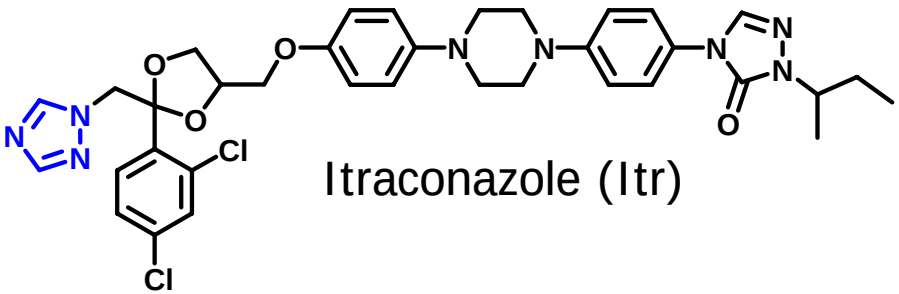
In vitro Activity of Azoles



Fluconazole (Flu)



Voriconazole (Vor)



Itraconazole (Itr)

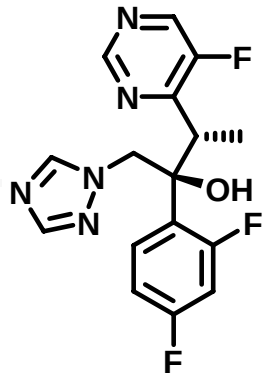
MIC (μg/mL)*

	Flu	Itr	Vor
Aspergillus fumigatus	>50	0.39	0.09
Candida albicans	1.00	0.12	0.03
Candida krusei	>25	0.05	0.24
Candida glabrata	1.90	0.19	0.19
Cryptococcus neoformans	9.6	0.39	0.39

*minimum inhibitory concentration

Dickinson R. F. *et al*. *Bioorg*. *Med*. *Chem*. *Lett*. **1996**, *6*(16), 2031.

Voriconazole



- α-CH_3 gives a marked increase in activity
- Pyrimidine ring expands therapeutic window
- Side effects
- Multiple drug-drug interactions

Dickinson R. F. *et al*. *Bioorg*. *Med*. *Chem*. *Lett*. **1996**, *6*(16), 2031.
Ghannoum, M. A.; Rice L. B. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(4), 501.

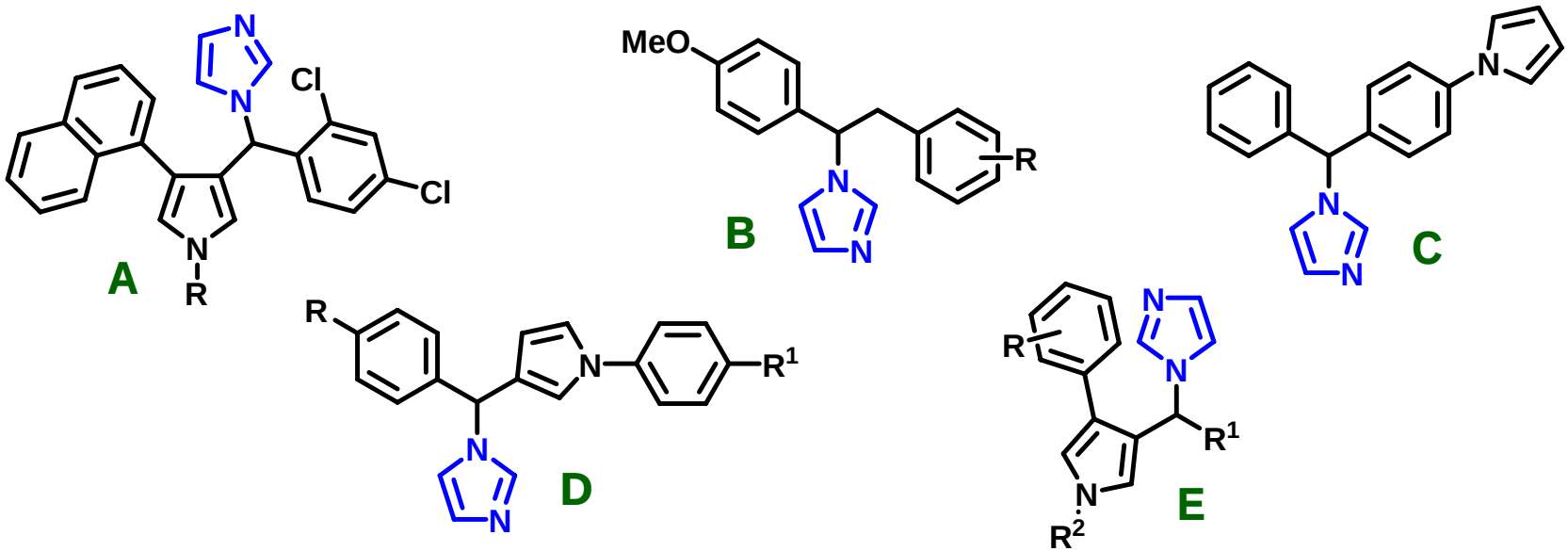
Drug-Drug Interactions

Rifampin
Rifabutin
Phenytoin
HIV Protease Inhibitors
NNRTIs
Cisapride
Quinidine
Cyclosporine
Tacrolimus
Omeprazole
Vinca Alkaloids
HMG-CoA Reductase Inhibitors
Sulfonylurea Oral Hypoglycemics
Dihydropyridine Calcium Channel Blockers
Efavirenz
Barbiturates
Terfenadine
Astemizole
Sirolimus
Pimozide
Ergot Alkaloids
Methadone
Warfarin
Benzodiazepine

Pfizer Inc. VFEND® Complete Product Information, March 2005.

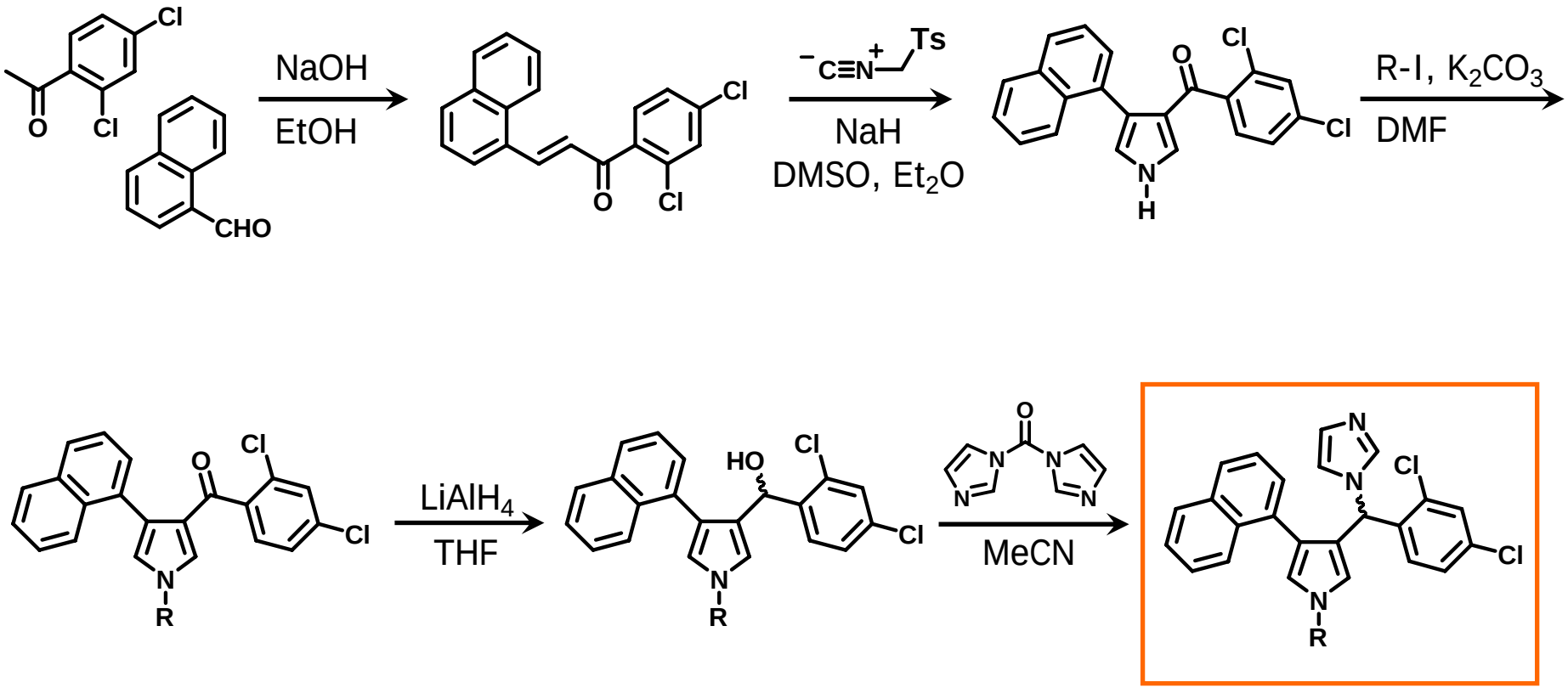
Quantitative SAR Study

- No 3-D structural data available in *Candida*
- Homology and pharmacophore modeling
- 5 structure classes: A–E



Di Santo R. *et al*. *J*. *Med. Chem*. **2005**, *48*, 5140

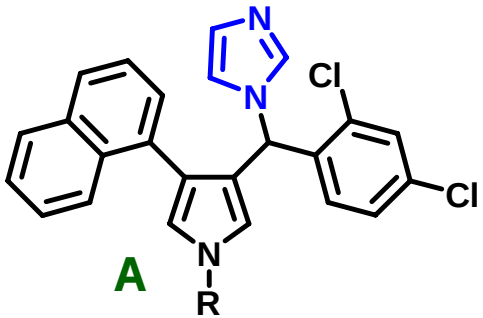
Synthesis of Class A



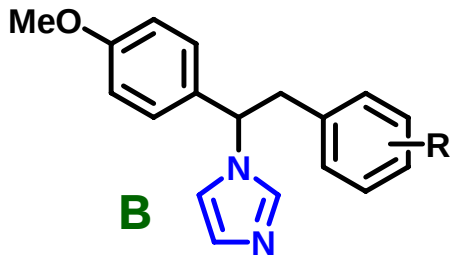
Di Santo R. *et al*. *J. Med. Chem*. **2005**, *48*, 5140

In Vitro Anti-*Candida* Activity

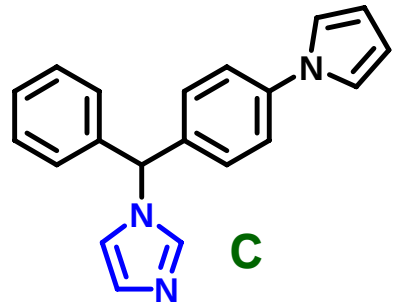
- Tested in 12 *Candida albicans* strains



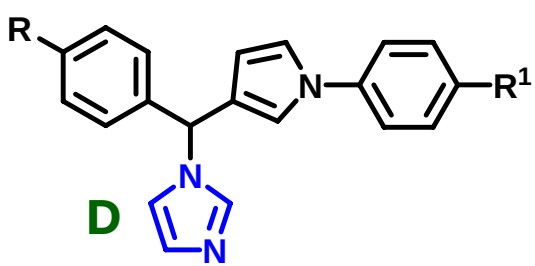
MIC = 0.74–3.9 μg/mL



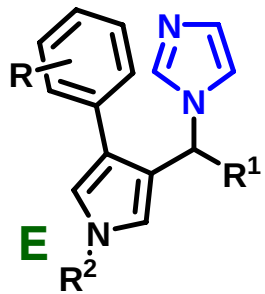
3.5–340 μg/mL



24 μg/mL



2.5–26 μg/mL

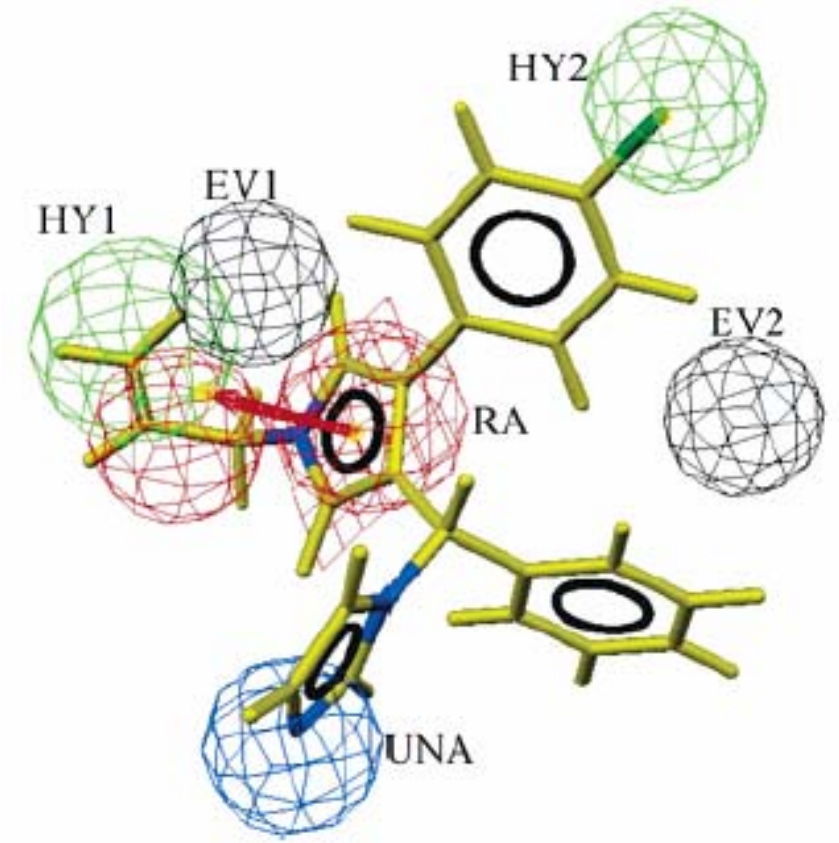


0.07–220 μg/mL

Fluconazole
0.24 μg/mL

Di Santo R. *et al*. *J*. *Med. Chem*. **2005**, *48*, 5140

Pharmacophore Generation

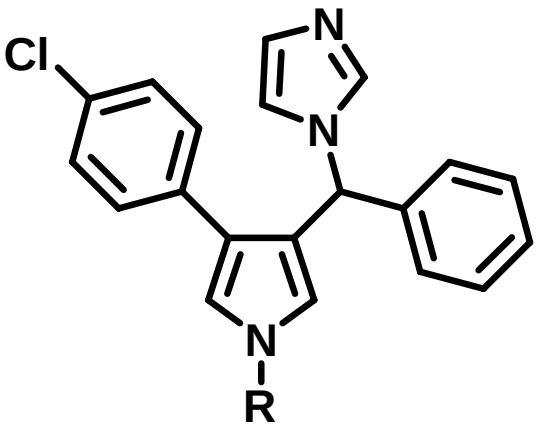


- Training set: Classes A–E activities spanned 4 orders of magnitude (n=24, r^2=0.93)
- Whole set (n = 64, r^2 = 0.73)
- The most active compounds matched all pharmacophore features
  - All from Class E
- Fluconazole matched 3 of 4

UNA = unsubstituted Ar N **EV** = excluded volumes
HY = hydrophobic **RA** = aromatic ring

Di Santo R. *et al*. *J. Med. Chem*. **2005**, *48*, 5140

Activity Prediction

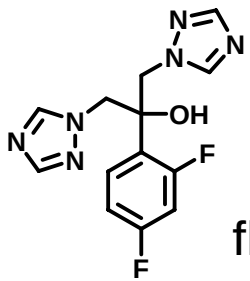


Class E

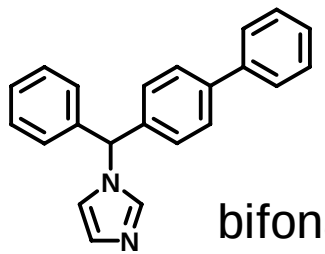
Cmpd	X	Expt	Calc	Error
1	CH_3	0.025	0.13	5.1
2	C_3H_7	0.023	0.0064	-3.6
3	CH_2-C_3H_5	0.025	0.052	2.1
4	$CH{=}CH_2$	0.031	0.26	8.3
5	$CH_2CH{=}CH_2$	0.019	0.0076	-2.5
6	$CH_2CH{=}(CH_3)_2$	0.043	0.063	1.5
Flu		0.069	0.59	8.6

Values expressed as MIC_{cmpd}/MIC_{bif}

Calc/ Expt



fluconazole

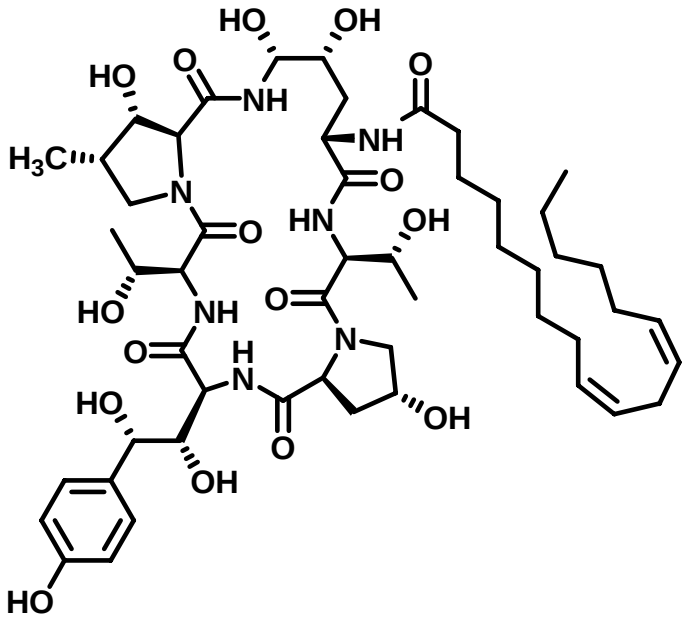


bifonazole

Di Santo R. *et al*. *J. Med. Chem*. **2005**, *48*, 5140

Azole Summary

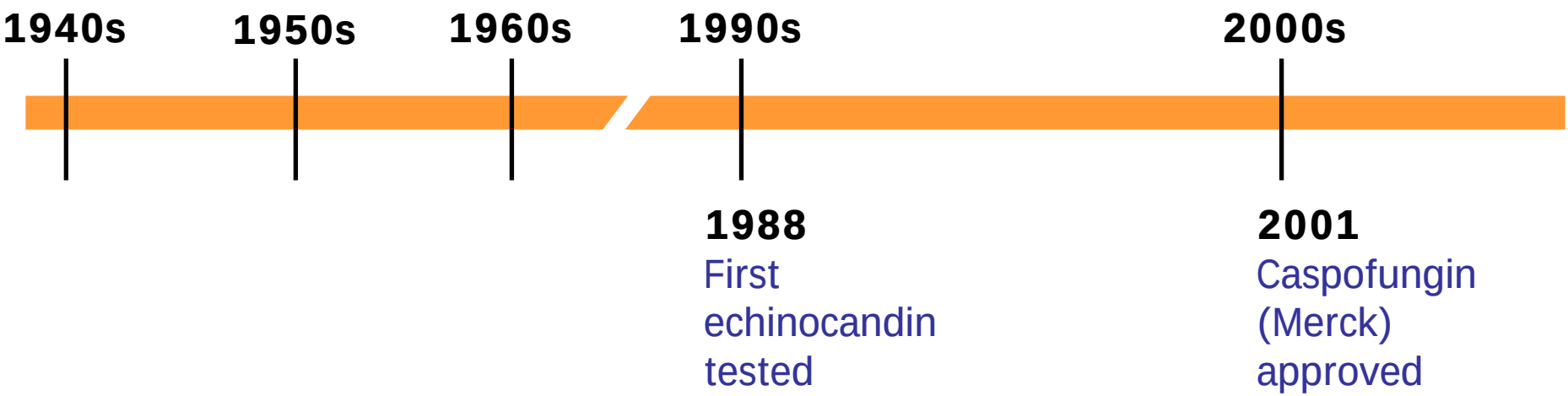
- 2nd generation targets resistant strains
- Broad spectrum activity
- Far less toxic than amphotericin B
- Multiple drug-drug interactions
- Fungistatic



Echinocandins

Targeting the fungal cell wall

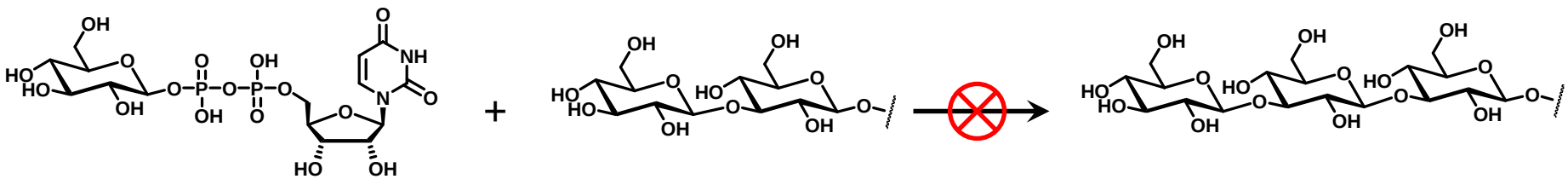
Key Events for Echinocandins



Sheehan, D. J. *et al*. *Clin*. *Microbiol*. *Rev*. **1999**, *12*(1), 40

Mechanism of Action

- Non-competitive inhibitors of β(1,3)-glucan synthase



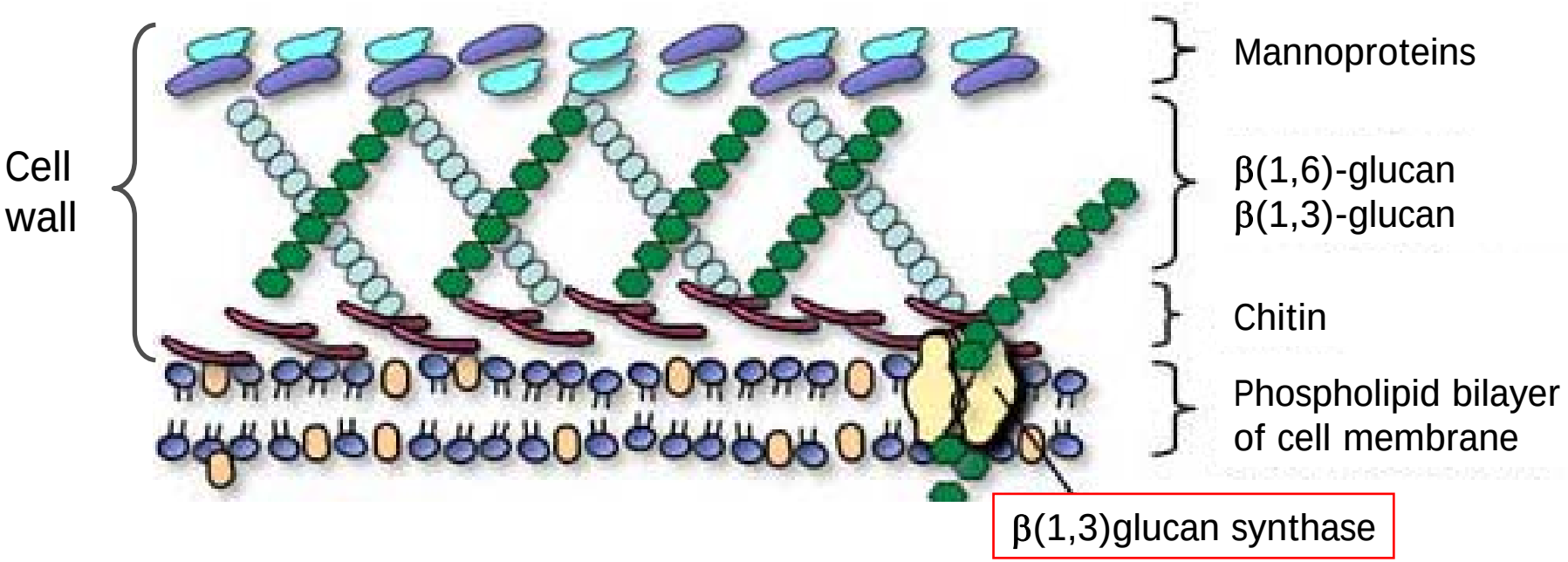
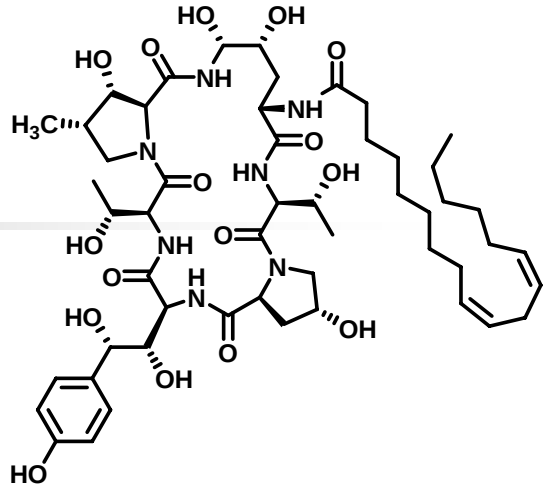


Image from DoctorFungus Corporation
Sawistowska-Schroder E. T. *et al*. *FEBS Lett.* **1984**, *173*(1), 134.

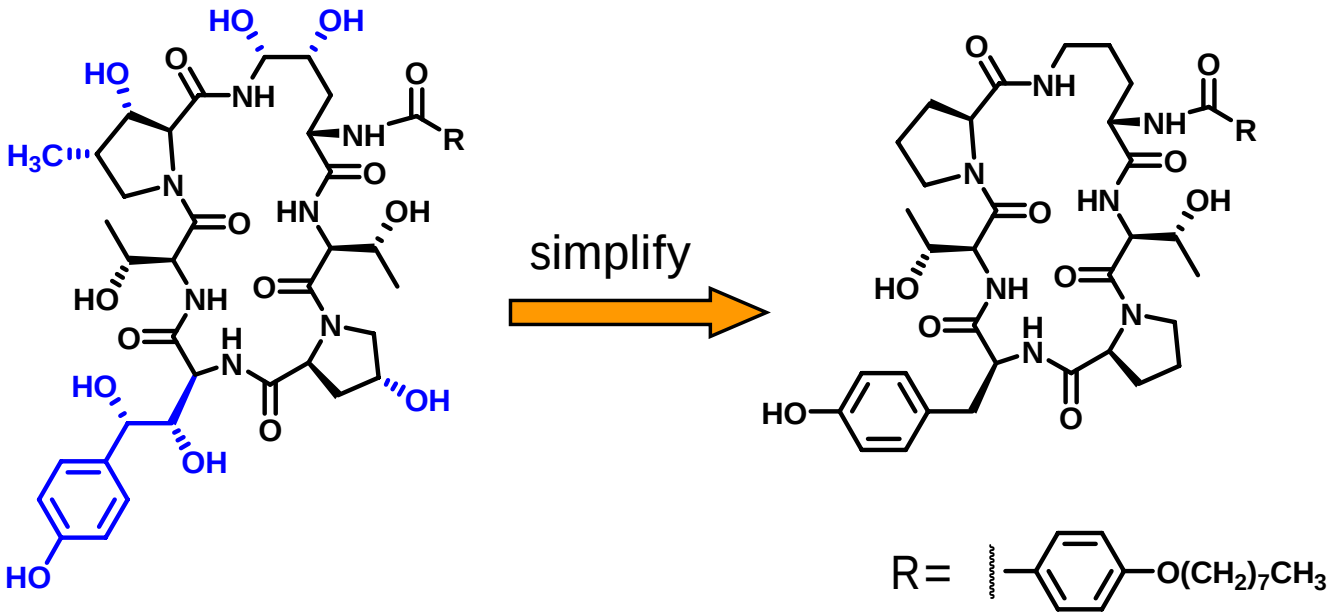
Echinocandins



- Fungicidal
  - Causes rapid lysis in growing cells
- *Candida* & *Pneumocystis carinii* activity
- Fewer drug-drug interactions
- Three in clinical development:
  - Caspofungin, micafungin, anidulafungin

Letscher-Bru, V.; Herbrecht R. *J. Antimicrob. Chemother.* **2003**, *51*, 513.

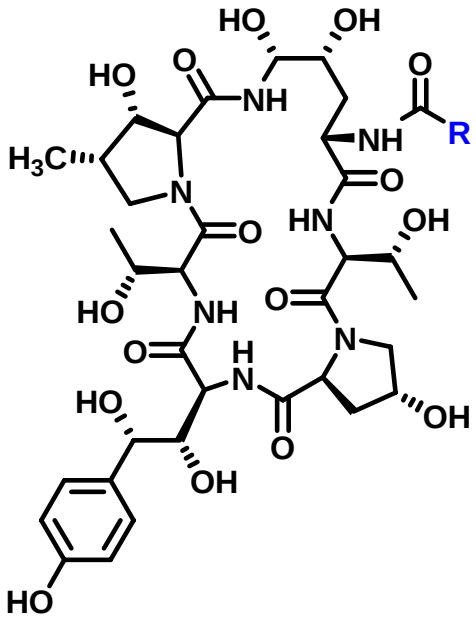
SAR of Simplified Analogs

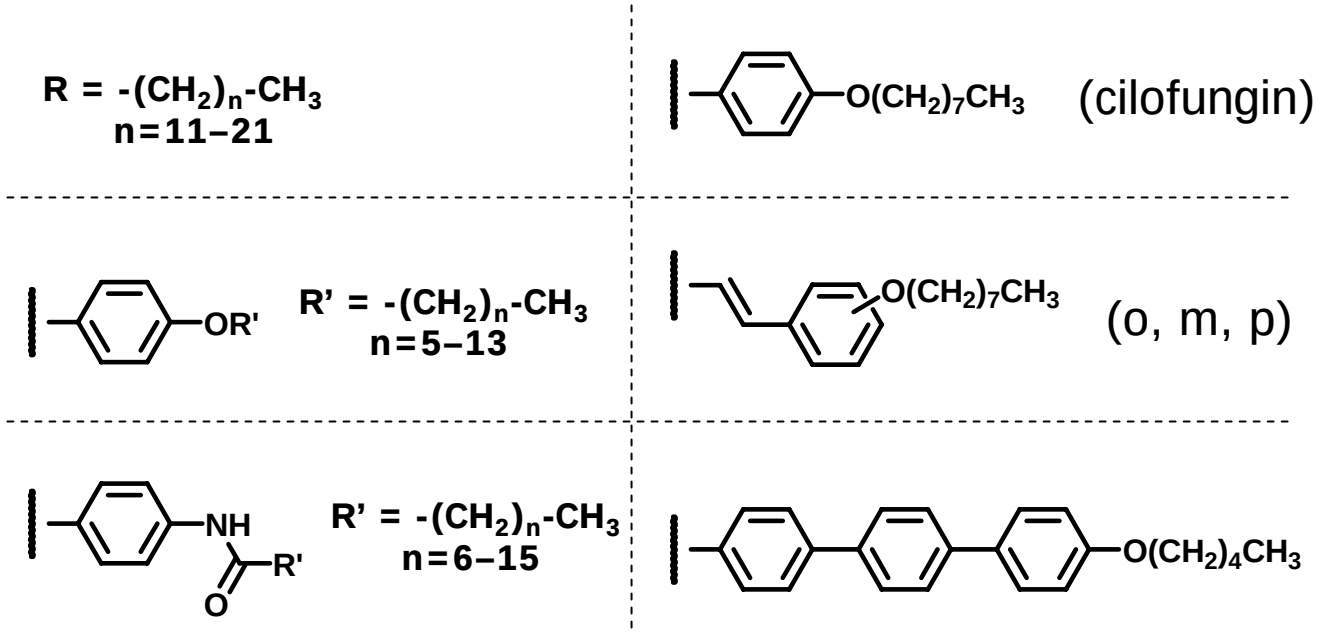


- Replaced unusual amino acids
  - L-homotyrosine crucial for antifungal activity
  - L-threonine could replace 3-hydroxy-4-methyl proline

Zambias R. A. *et al*. *J*. *Med*. *Chem*. **1993**, *35*, 2843

Sidechain SAR Study



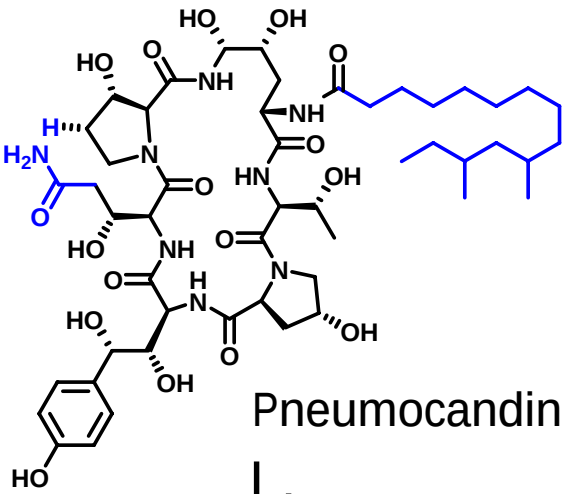


- Too long: hemolytic *in vitro*
- Too short: no antifungal activity
- C log P > 3.5 = antifungal

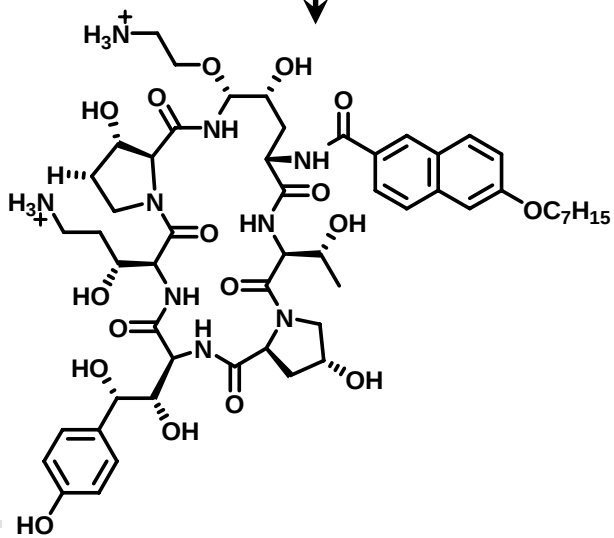
Debono J. *et al*. *J*. *Med*. *Chem*. **1995**, *38*, 3271

Cationic Derivatives

- Cilofungin withdrawn due to toxicity of solubilizing agent
- Increase water solubility
- Unique regio-, chemo-, and stereoselective synthesis from core
  - 4 linear steps
  - 83% yield



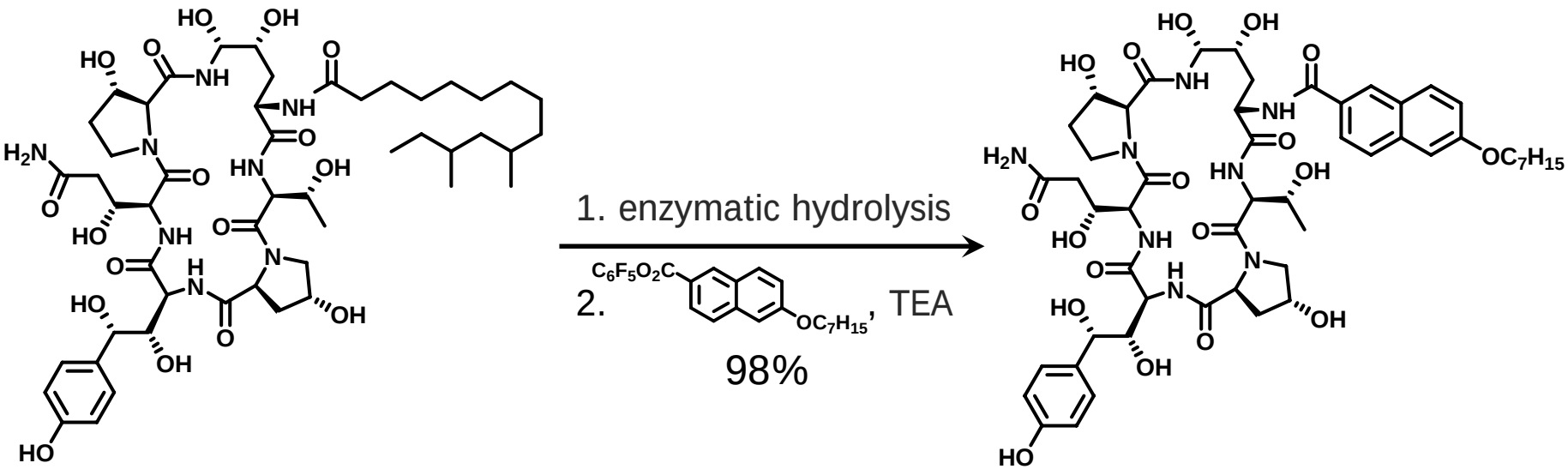
Pneumocandin B



Bouffard, F. A. *et al*. *J*. *Med*. *Chem*. **1994**, *37*, 222.
Journet, M. *et al*. *J. Org. Chem.* **1999**, *64*, 2411.

Pneumocandin Semi-Synthesis

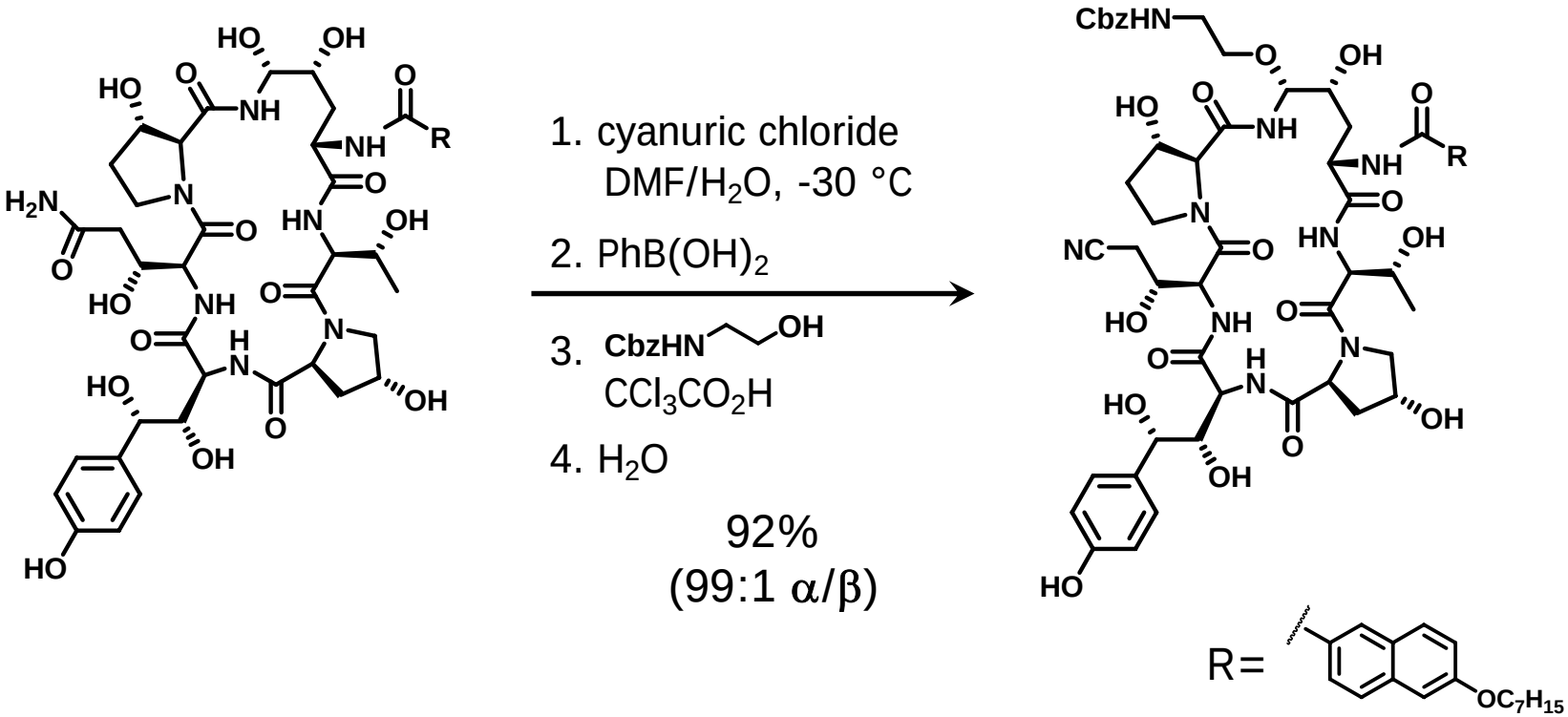
- Pneumocandin B_o isolated from *Glarea lozoyensis*
- Most efficient route began with acylation of amine



Journet, M. *et al*. *J. Org. Chem.* **1999**, *64*, 2411.

Dehydration and Etherification

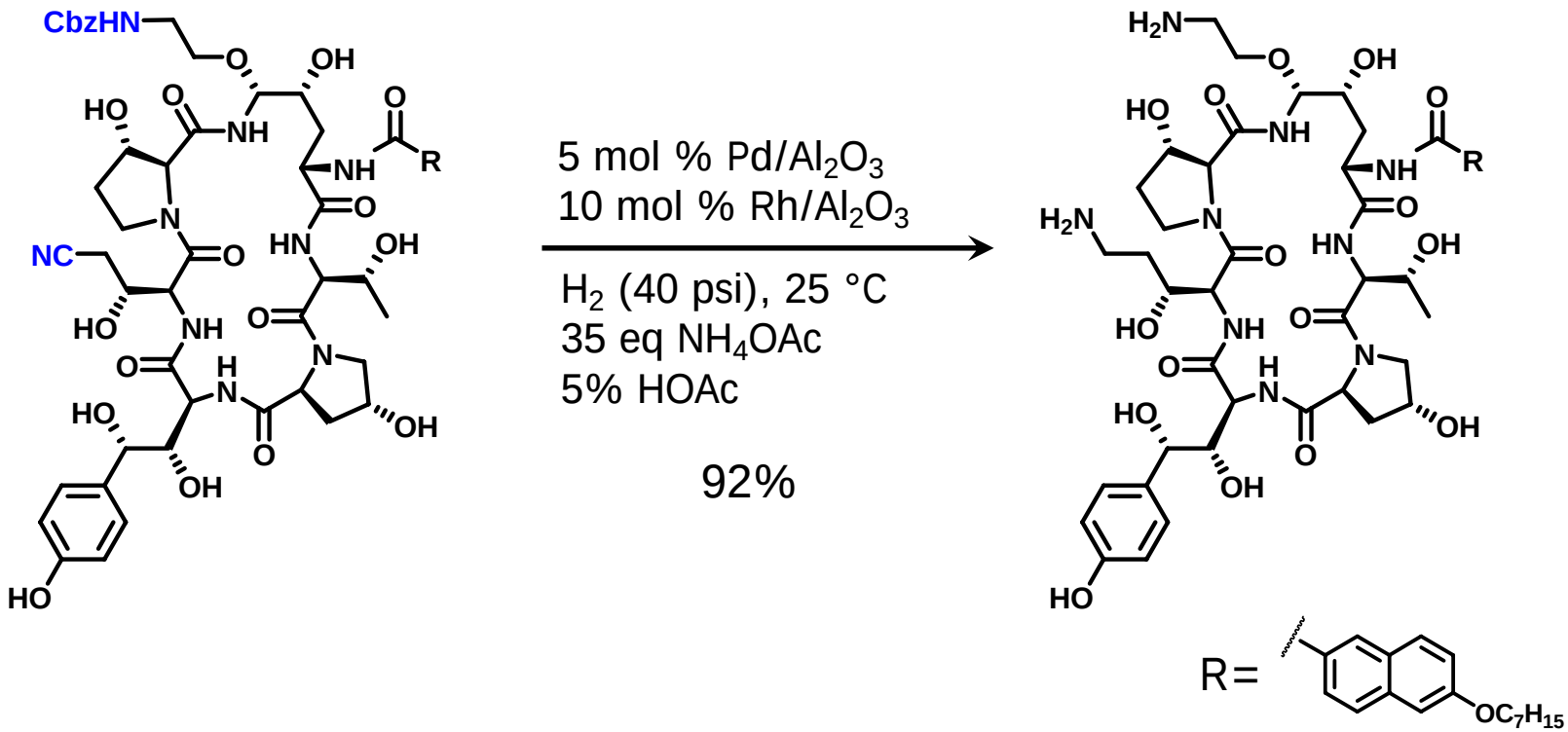
- Direct reduction of amide gave mixture of products
- Protection of benzylic alcohol required



Journet, M. *et al*. *J. Org. Chem.* **1999**, *64*, 2411.

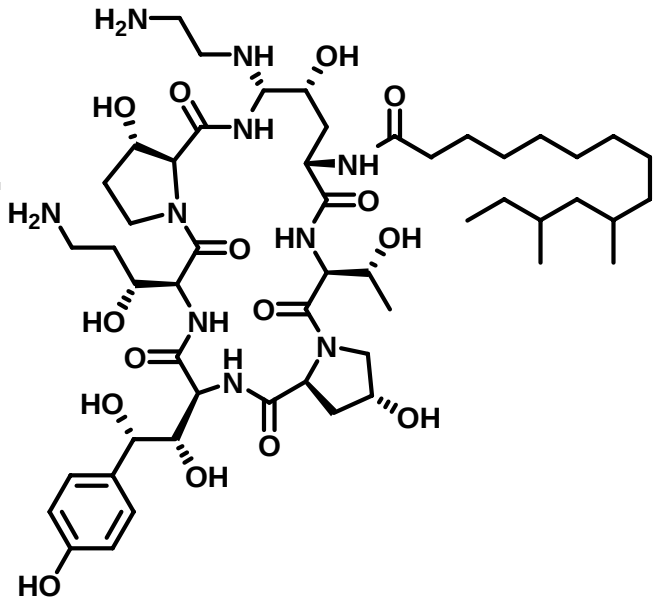
One Pot Hydrogenation

- Hydrogenation of nitrile
- Deprotection of Cbz-protected amine



Journet, M. *et al*. *J. Org. Chem.* **1999**, *64*, 2411.

Caspofungin

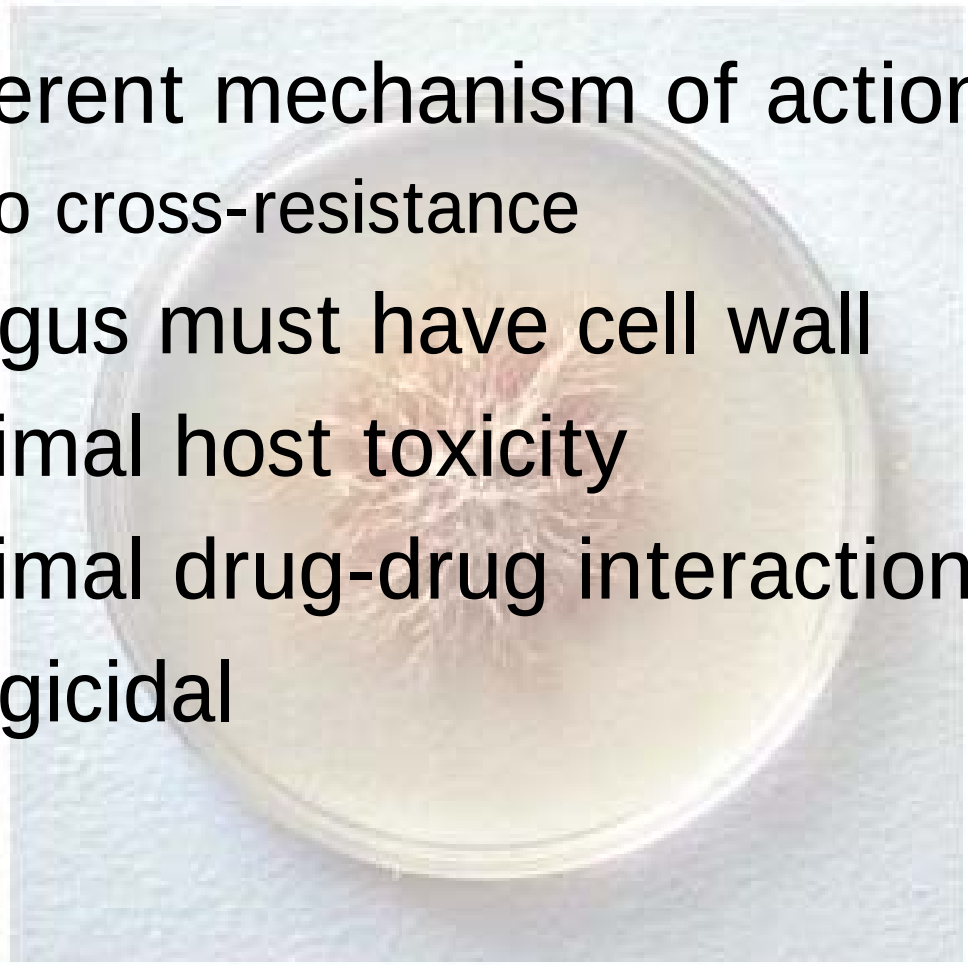


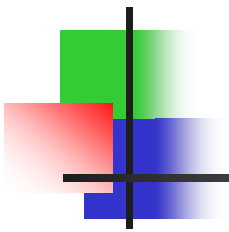
- Semi-synthetic, fungal fermentation product
  - *Glarea lozoyensis*
- Approved in 2001 for invasive aspergillosis
  - Resistant to amphotericin B or triazole failure
  - Synergy: weakens cell wall and allows passage of amphotericin B or fluconazole
- 2002 for esophageal candidiasis

Groll A. H.; Walsh T. J. *Swiss Med. Wkly.* **2002**, *132*, 303.

Echinocandin Summary

- Different mechanism of action
  - No cross-resistance
- Fungus must have cell wall
- Minimal host toxicity
- Minimal drug-drug interactions
- Fungicidal

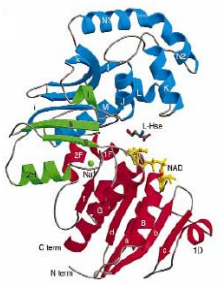




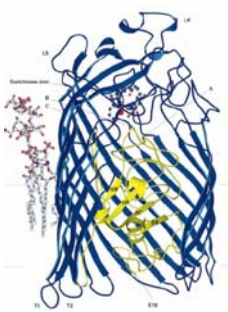
Future Targets

Moving into the cell

Promising Future Targets



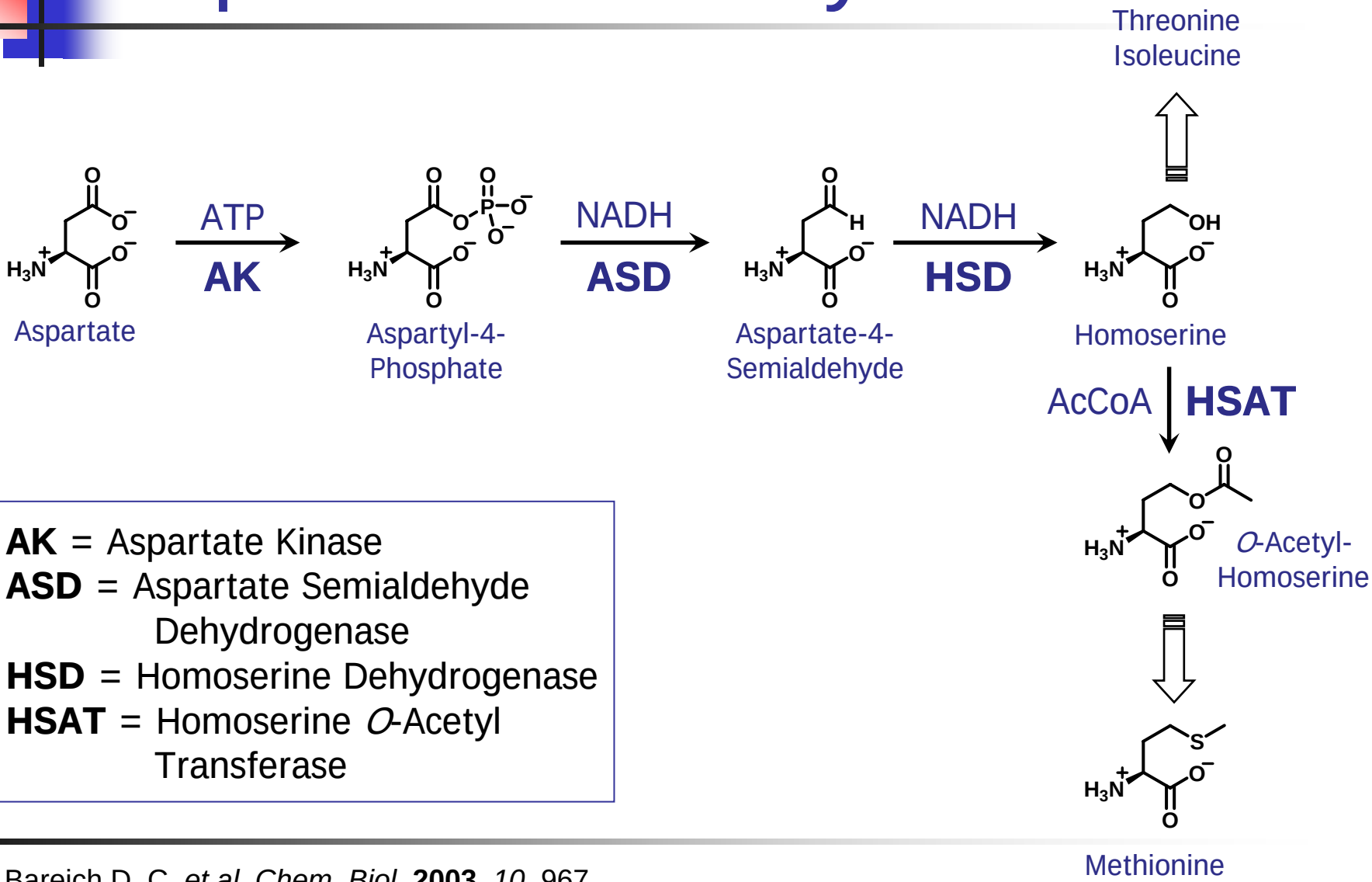
- Aspartate pathway
  - Fungi must synthesize Met, Ile, Thr



- Siderophore biosynthesis
  - Iron importation mechanism

DeLaBarre B. *et al*. *Nat. Struct. Biol.* **2000**, *7*(3), 238.
Ferguson A. D. *et al*. *Science* **1998**, *282*, 2215.

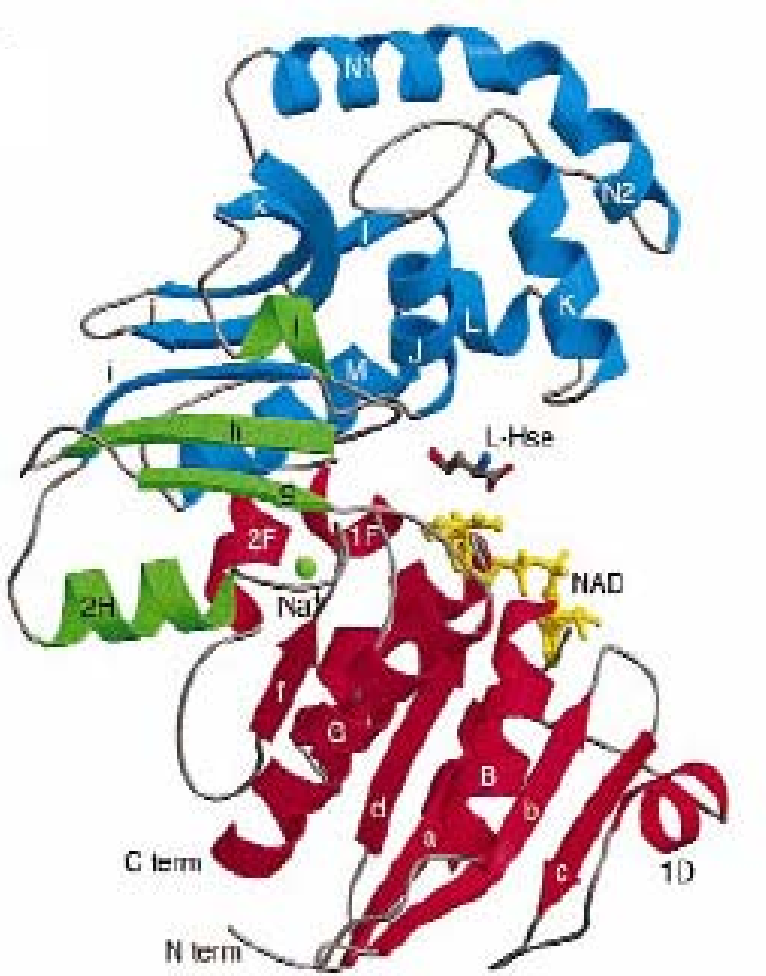
Aspartate Pathway

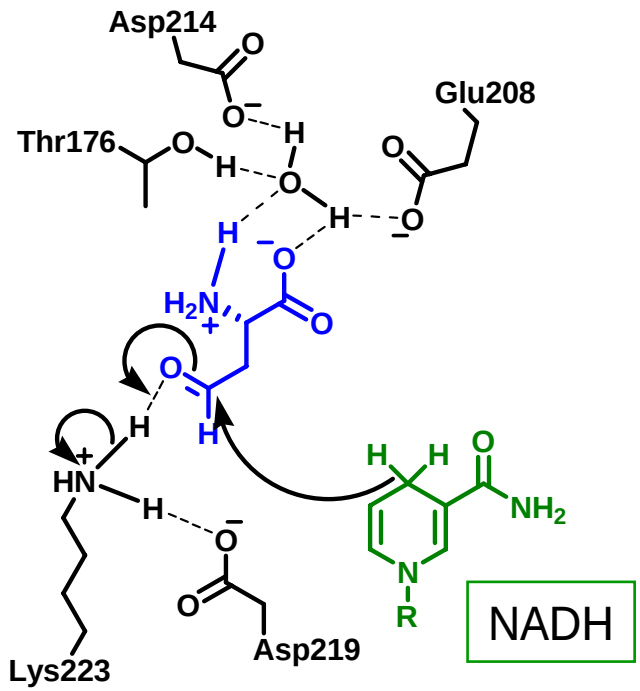


AK = Aspartate Kinase
ASD = Aspartate Semialdehyde Dehydrogenase
HSD = Homoserine Dehydrogenase
HSAT = Homoserine *O*-Acetyl Transferase

Bareich D. C. *et al*. *Chem. Biol.* **2003**, *10*, 967.

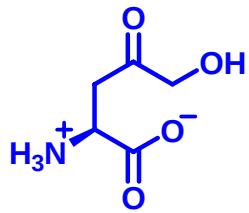
Homoserine Dehydrogenase





DeLaBarre B. *et al*. *Nat. Struct. Biol.* **2000**, *7*(3), 238.

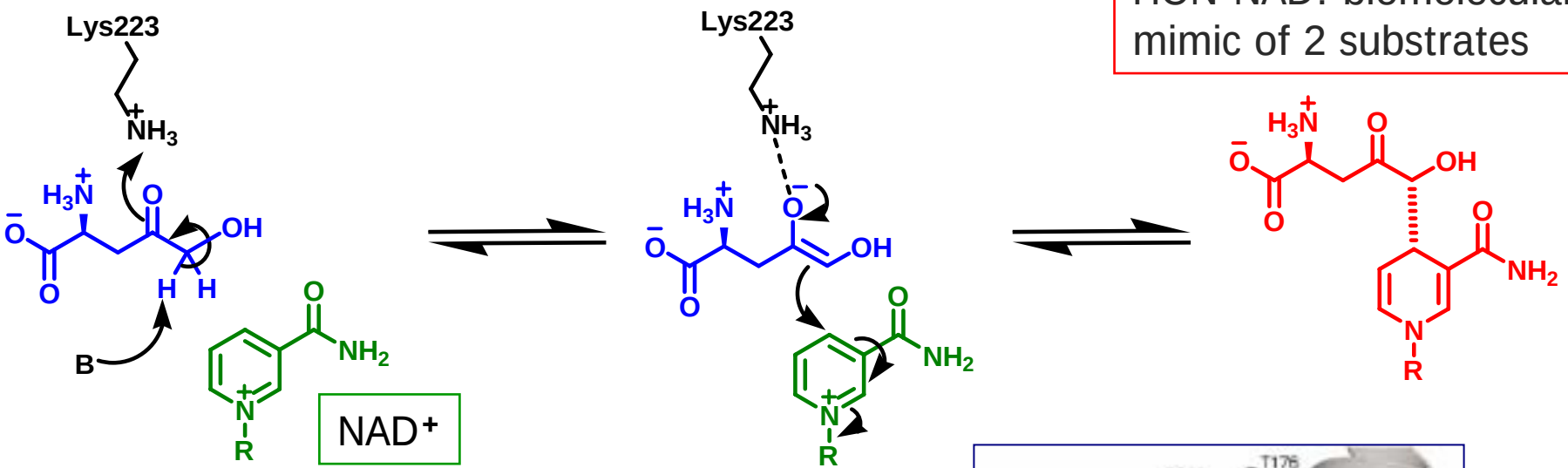
Natural Product Inhibitor

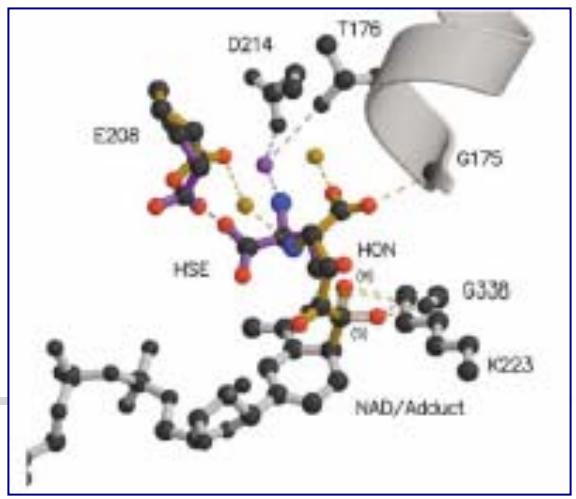


- Promising antifungal: 5-hydroxy-4-oxonorvaline (HON)
  - Isolated from *Streptomyces* over 40 yrs ago
  - Active against *Cryptococcus* and *Candida*
  - 100% survival in rats, no toxicity
- K_i = 2 mM; yet capable of arresting cell growth (irreversible)

Jacques S. L. *et al*. *Chem. Biol.* **2003**, *10*, 989.

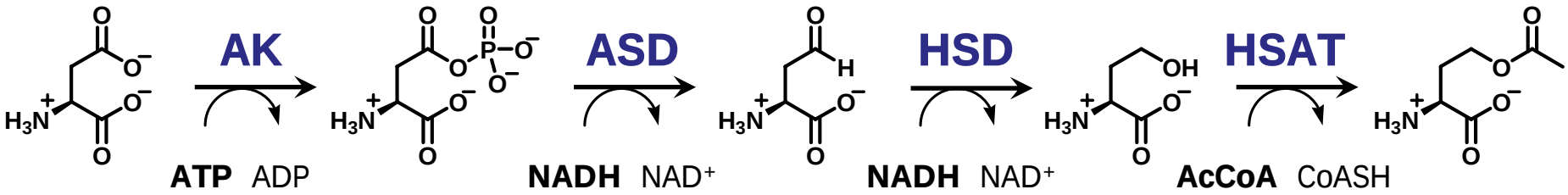
Mechanism of Inhibition

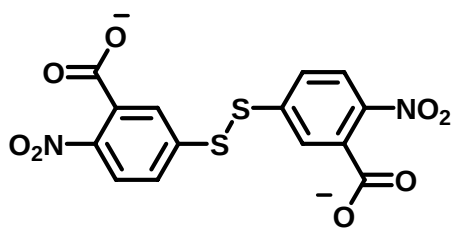


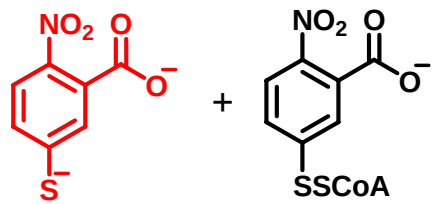


Jacques S. L. *et al*. *Chem. Biol.* **2003**, *10*, 989.

Coupled Assay





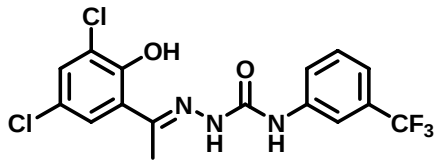
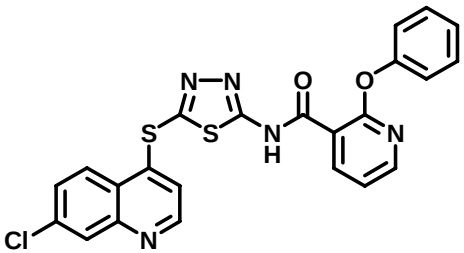
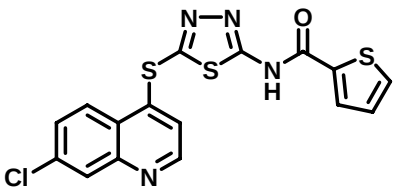
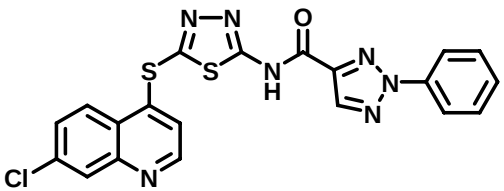


AK = Aspartate Kinase
ASD = Aspartate Semialdehyde Dehydrogenase
HSD = Homoserine Dehydrogenase
HSAT = Homoserine *O*-Acetyl Transferase

λ_{max} = 412 nM
ε = 13600 M^{-1} cm^{-1}

Bareich D. C. *et al*. *Chem. Biol.* **2003**, *10*, 967.

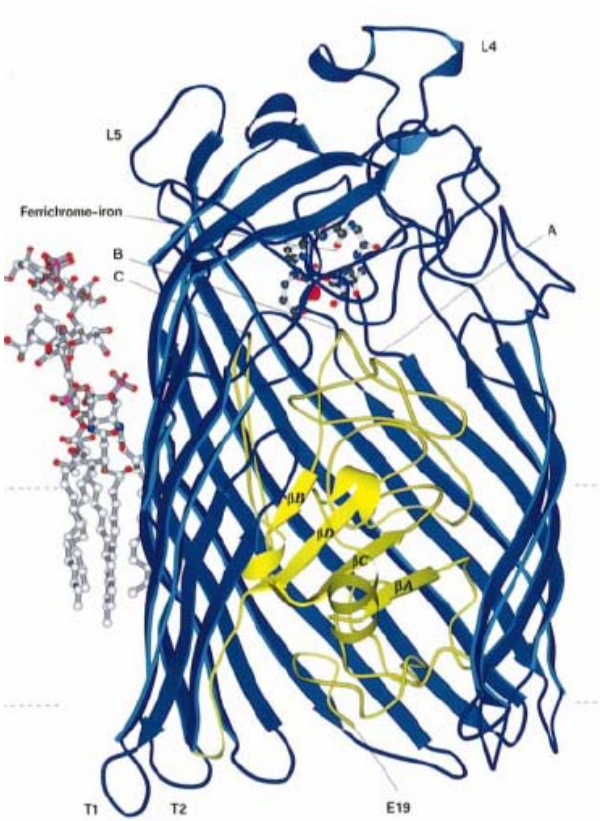
Novel Inhibitors of AK

	IC_{50} (μM)
 **1**	18 ± 3.7
 **2**	3.1 ± 0.8
 **2a**	3.6 ± 0.8
 **2b**	1.6 ± 0.7

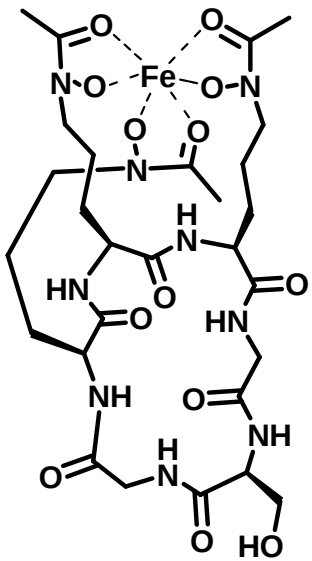
- Reversible inhibitors
- First non-amino acid inhibitors of fungal AK
- Leads to new compound development
- No effect on growth of Candida species
  - Membrane transport or efflux problems

Bareich D. C. *et al*. *Chem. Biol.* **2003**, *10*, 967.

Siderophore Function



Ferric-hydroxamate uptake (FhuA) protein



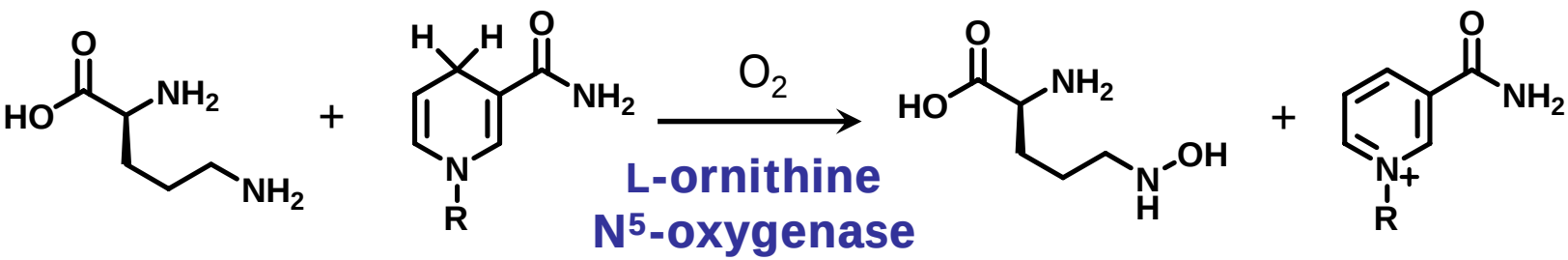
ferricrocin

- Fungi must scavenge for iron inside host
- Siderophores bind soluble iron with high affinity
- Actively transported through cell wall
- Couple antifungals to iron-binding motif

Ferguson A. D. *et al*. *Science* **1998**, *282*, 2215.
Winkelman G. *Biometals* **2002**, *30*(4), 691.

sidA Required for Virulence

- *sidA* encodes first committed step in hydroxamate siderophore biosynthesis
- Δ*sidA*: no growth in serum, no virulence in animal model
- Minimal host toxicity



Hissen, A. H. T. *et al*. *Infect*. *Immun*. **2005**, *73*(9), 5493.
Schrettl, M. *et al*. *J*. *Exp*. *Med*. **2004**, *200*, 1213.

Conclusions

- Invasive fungal infections remain a complication of modern medicine
- Urgent need exists for improved antifungal agents
- Extensive work is being done to validate new targets and develop new drugs

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- Jesse O'Neill

Allergic fungal sinusitis

Curvularae lunata

August 2002

1 week on amphotericin B

kidney failure

potassium levels

11 months on voriconazole

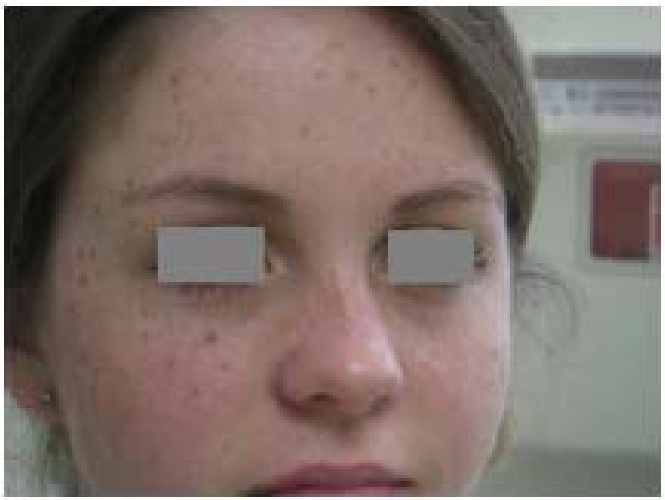

Fig 1. Numerous solar lentigines and ephelides on face 4 weeks after discontinuation of voriconazole treatment.

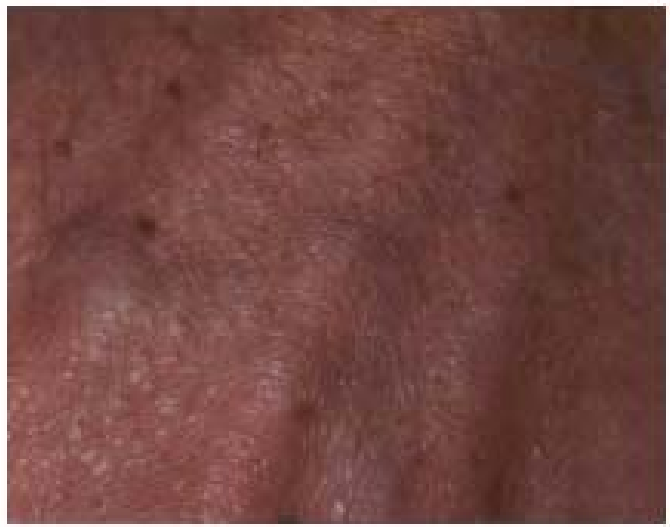

Fig 2. Solar elastosis and lentigines on back of left hand immediately after discontinuation of voriconazole treatment.

Racette A. J. *et al*. *J*. *Am*. *Acad*. *Dermatol*. **2005**, *52*(5), S81.