

Natural Products and the Quest For Novel Drugs

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(Slides available at www.chemtract.com under “Educational”)

Natural Products...Novel Drugs

Natural Product

1. Naturally occurring compounds that are end products of secondary metabolism
2. A term used commonly in reference to chemical substances found in nature that have distinctive pharmacological effects. Such a substance is considered a natural product even if it can be prepared by total synthesis.

Drug

1. A substance used in the diagnosis, treatment, or prevention of a disease or as a component of a medication (i.e. a distinctive pharmacological effect).
2. A chemical substance, such as a narcotic or hallucinogen, that affects the central nervous system, causing changes in behavior and often addiction.

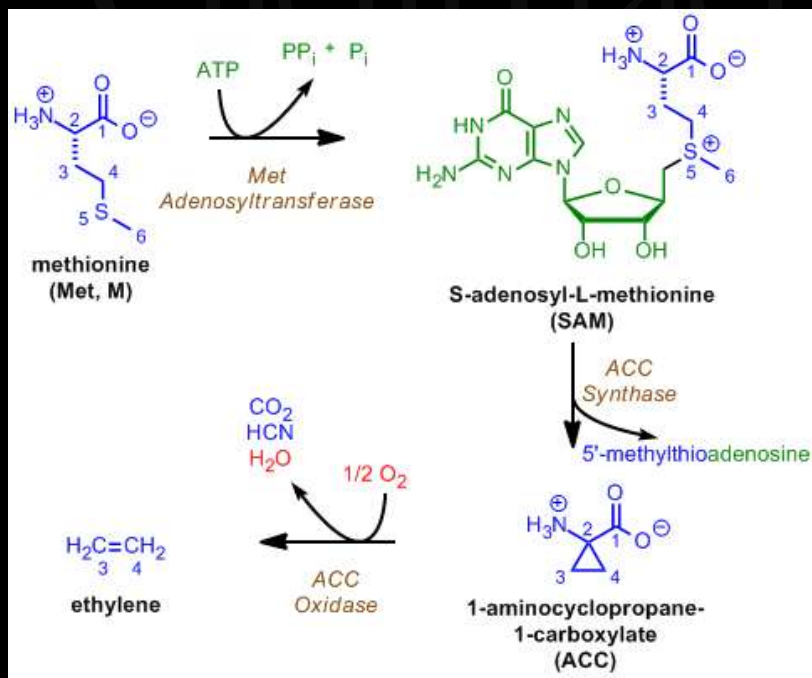
Sources of Natural Products

- All Domains of Life
 - Bacteria, Archaeobacteria and Eukaryotes (fungi and mammals)
- Some especially fruitful for drug discovery
 - Plants, bacteria and fungi
- Extraction – aqueous or organic solvents
- Purification – chromatography (HPLC)
- Identification – LCMS, NMR

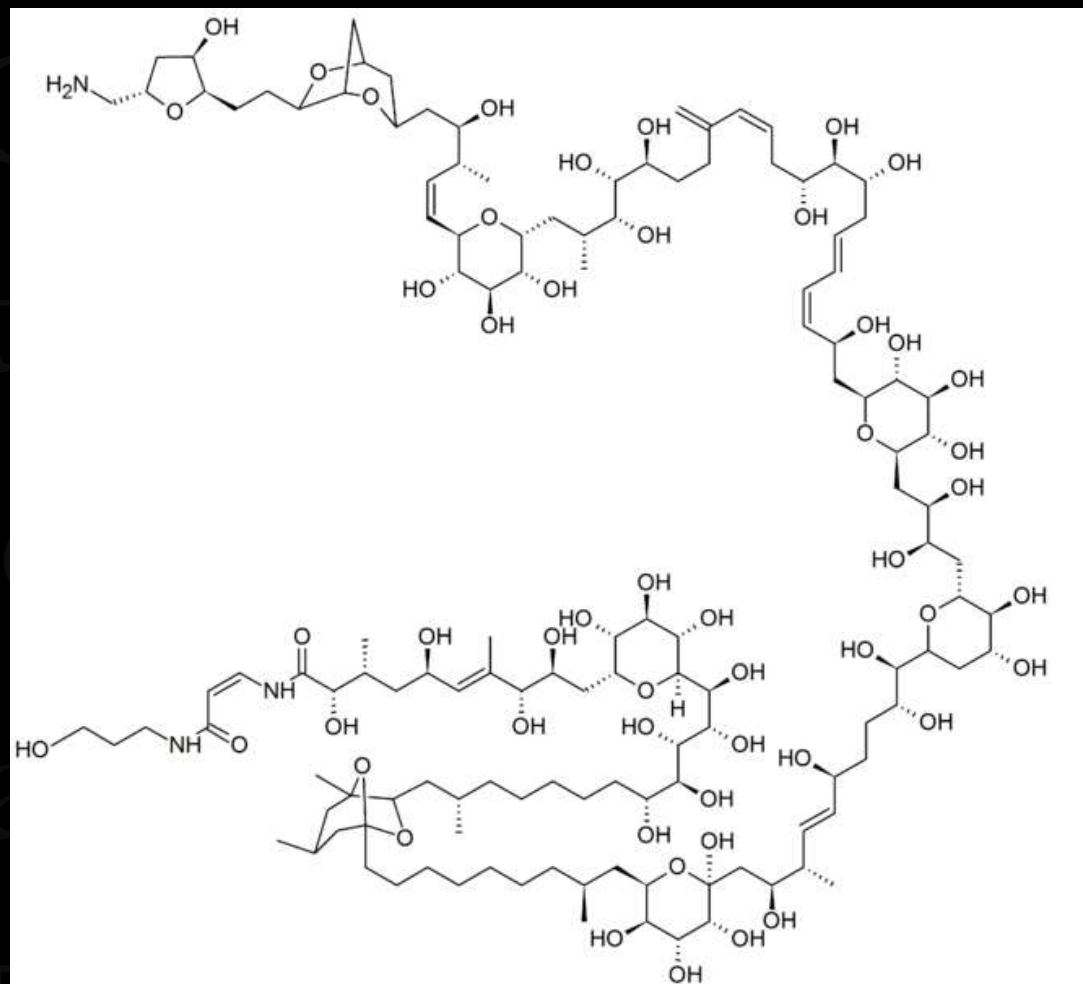
Why Are They Produced?

- Primary or Secondary Metabolism
- Waste Products (excretion, detoxification)
- Hormones (regulation of biochemical processes, communication)
- Defense (biological/chemical warfare)
- Improvement of Survival Fitness
 - D. Williams, *et al. J. Nat. Prod.*, **1989**, 52 (6), 1189–1208

Natural Products Can Be Simple or Complex



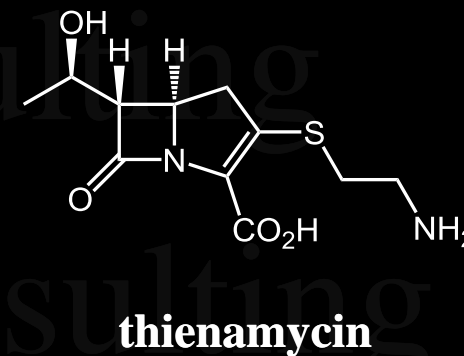
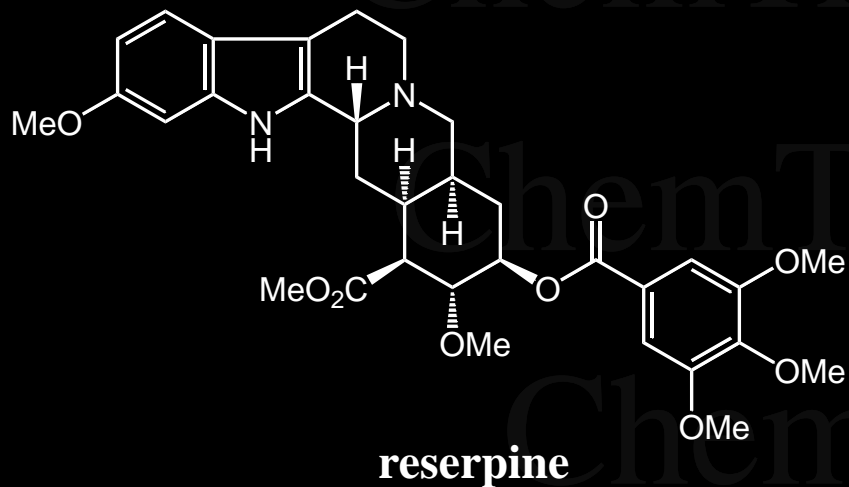
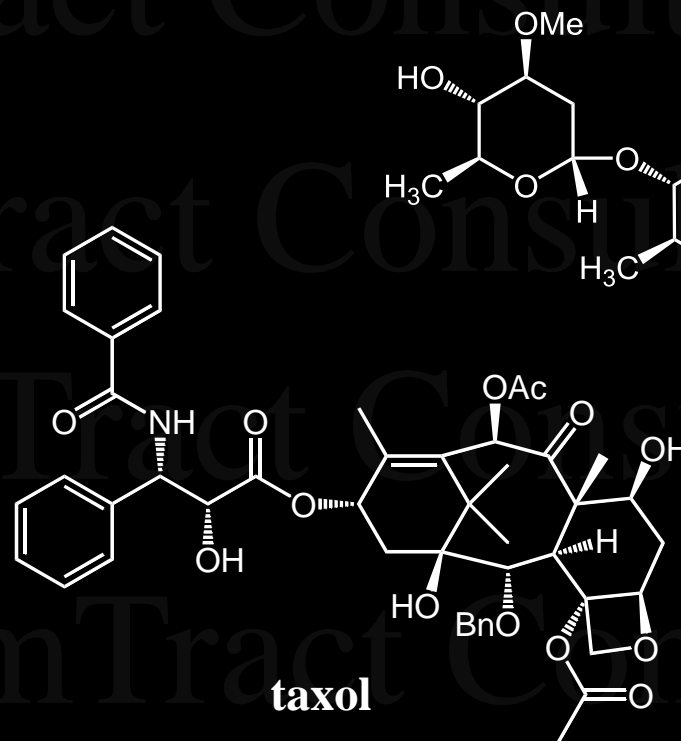
Even simple natural products have complex biosynthetic pathways



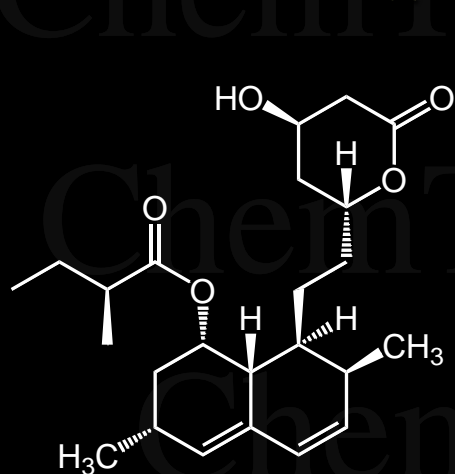
Palytoxin

one of the most toxic non-peptides known

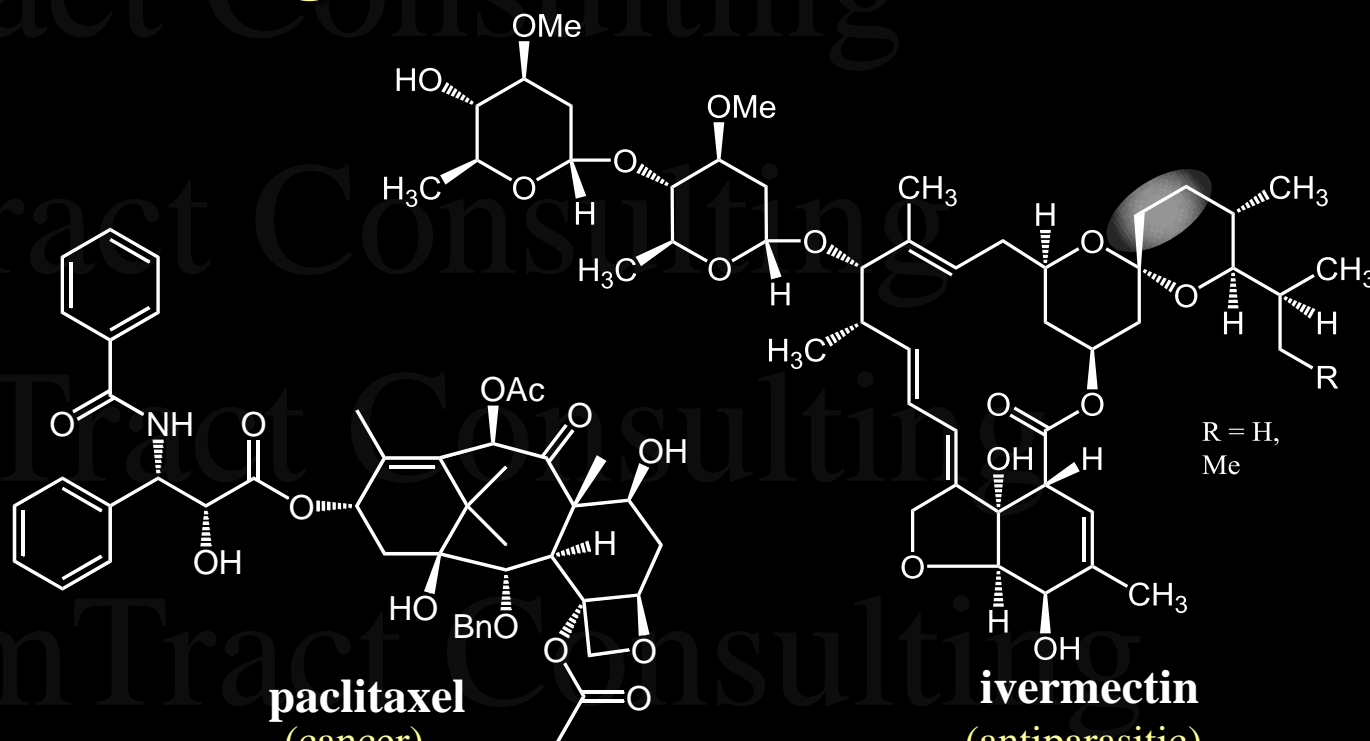
Examples of Natural Products



Examples of Drugs Derived From NPs

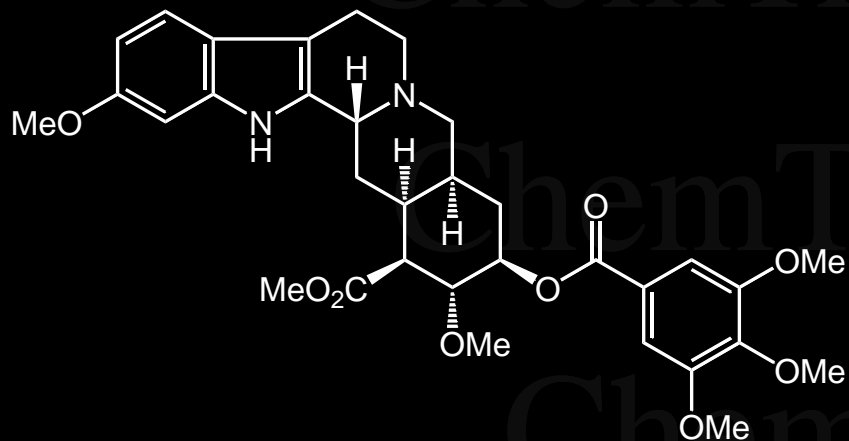


lovastatin
(elevated cholesterol)

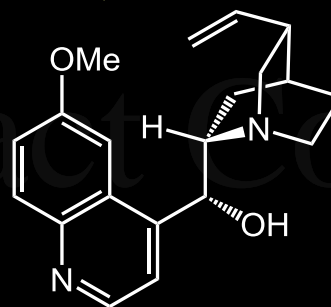


paclitaxel
(cancer)

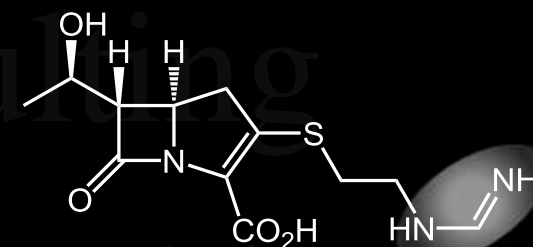
ivermectin
(antiparasitic)



reserpine
(hypertension)

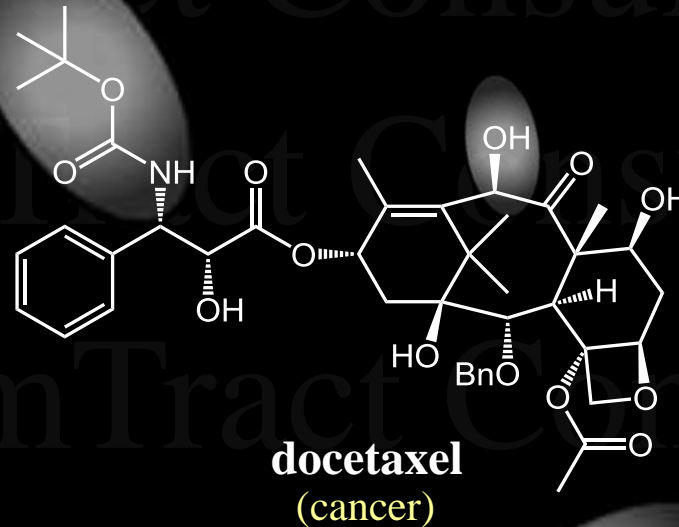
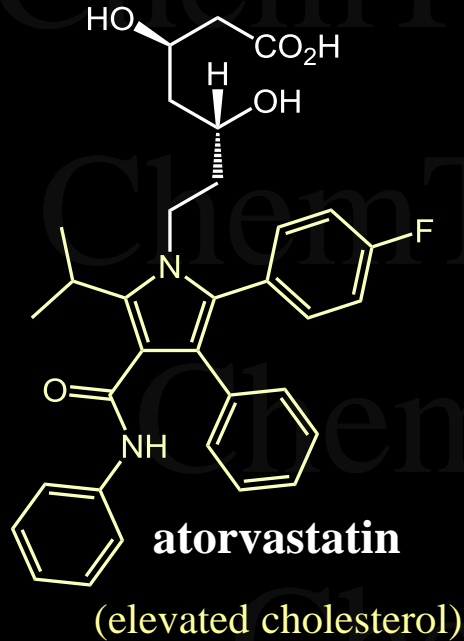


quinine
(malaria)



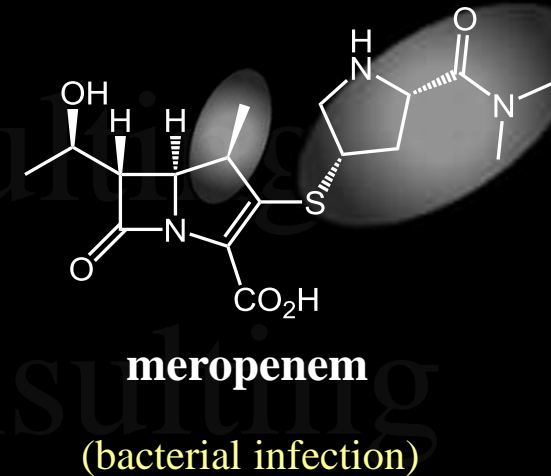
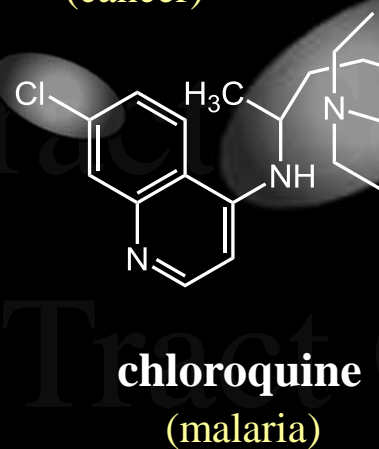
imipenem
(bacterial infection)

Second Generation Drugs

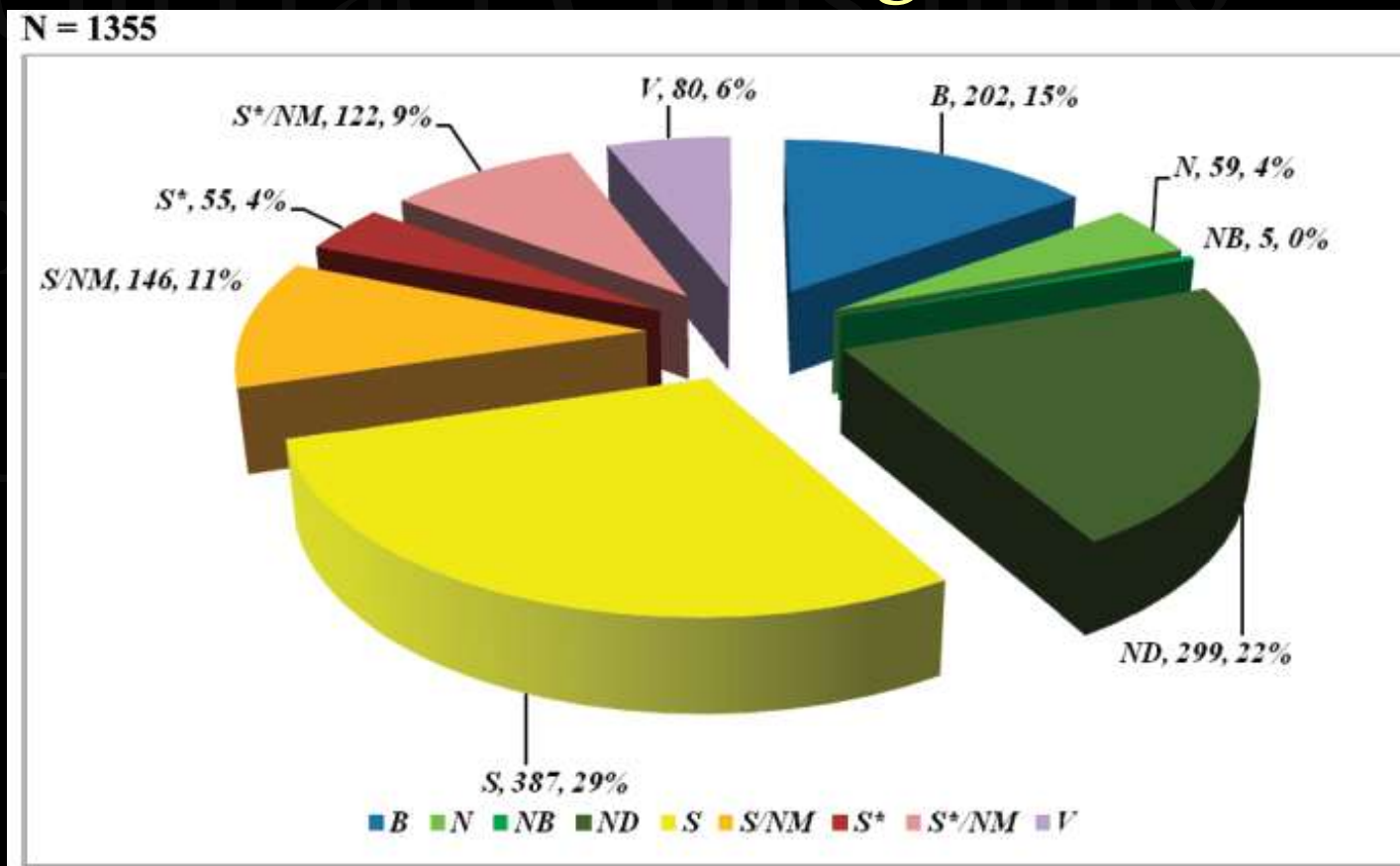


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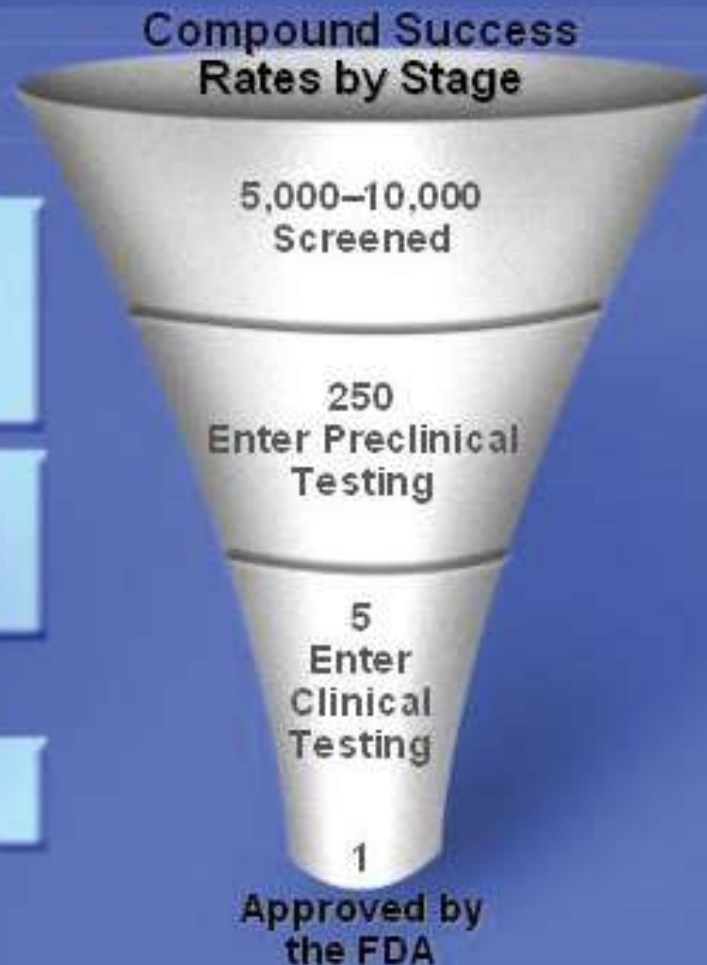
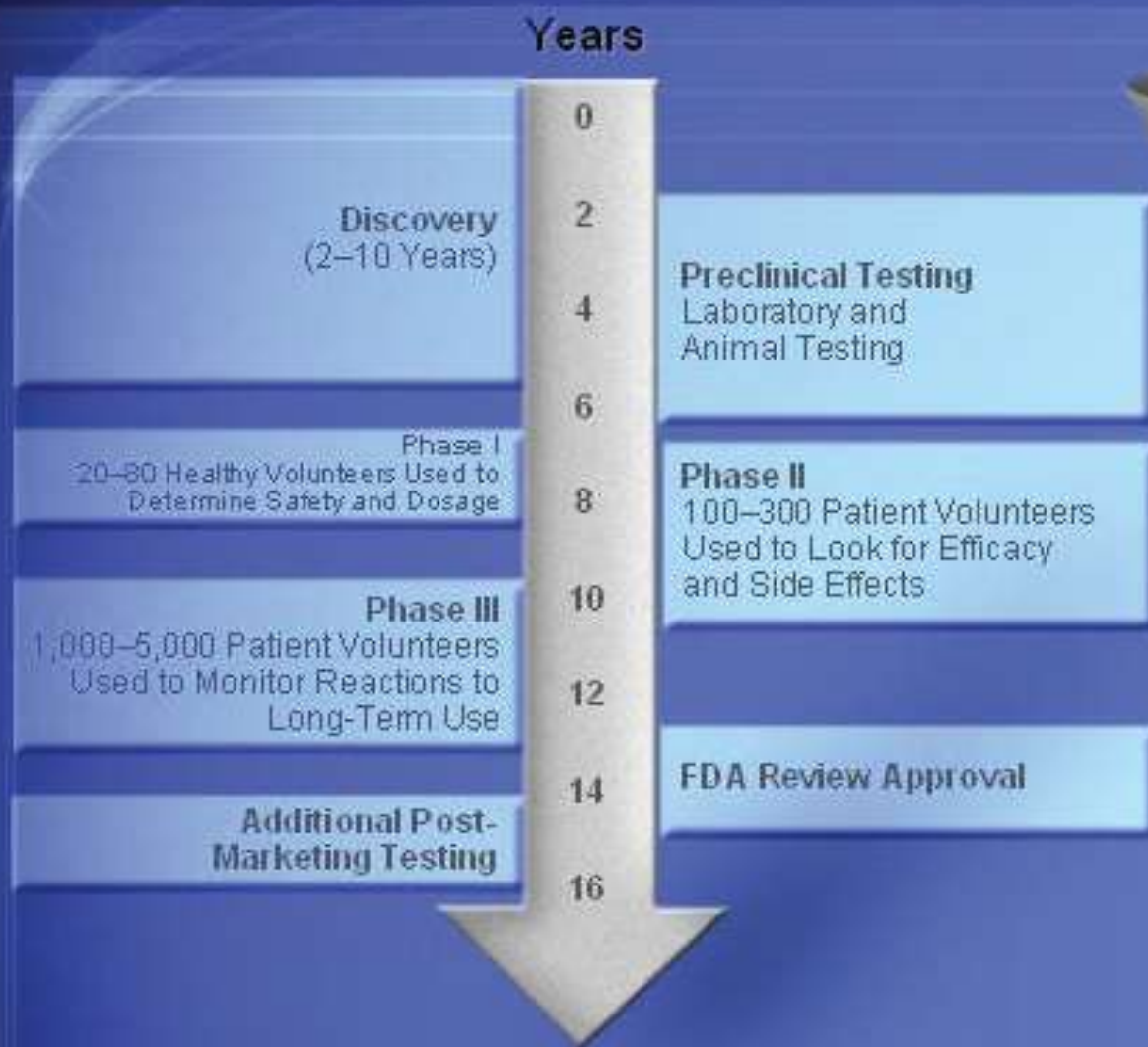


Sources of New Drugs 1981-2010



B = biological (peptide or protein), N = natural product, NB = natural product botanical, ND = natural product derivative, S = synthetic, S/NM = natural product mimic, S* = synthetic but pharmacophore is from natural product, S*/NM = synthetic with pharmacophore inspired by natural product, V = vaccine

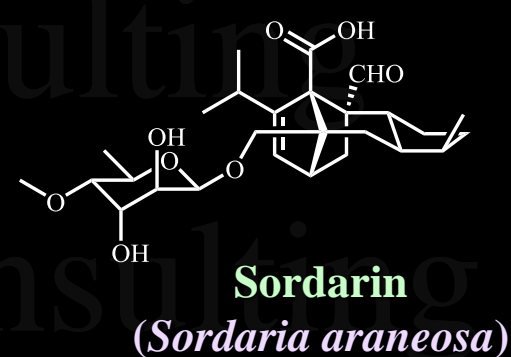
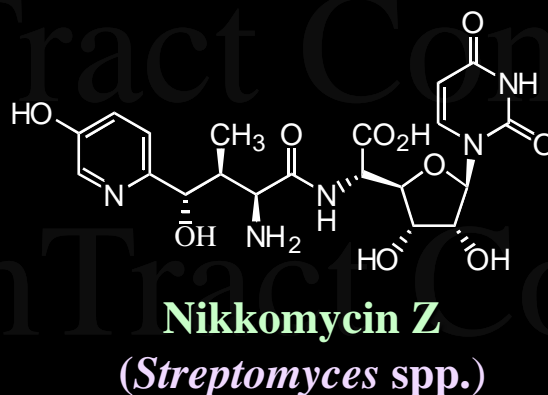
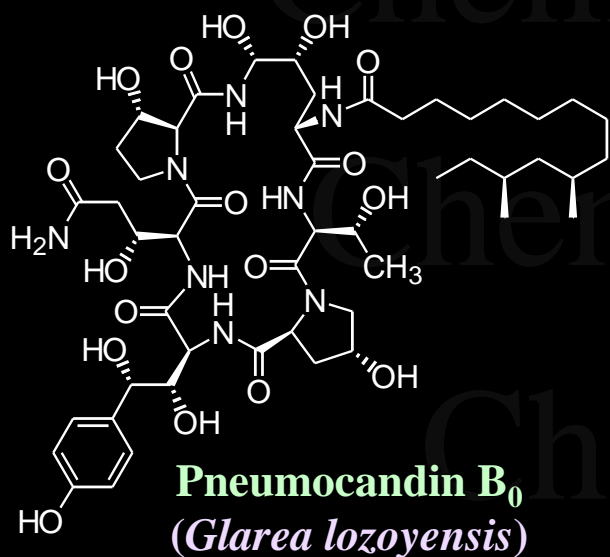
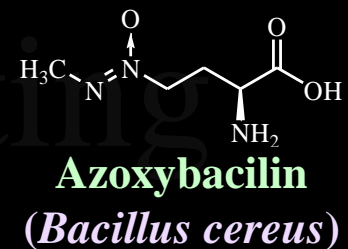
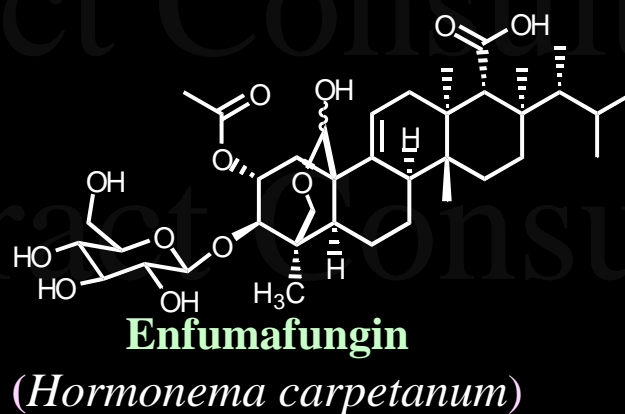
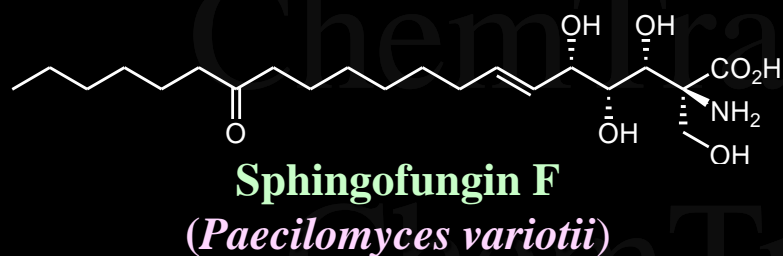
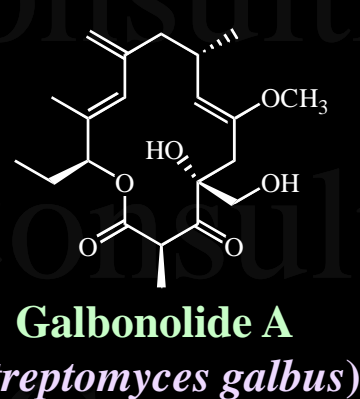
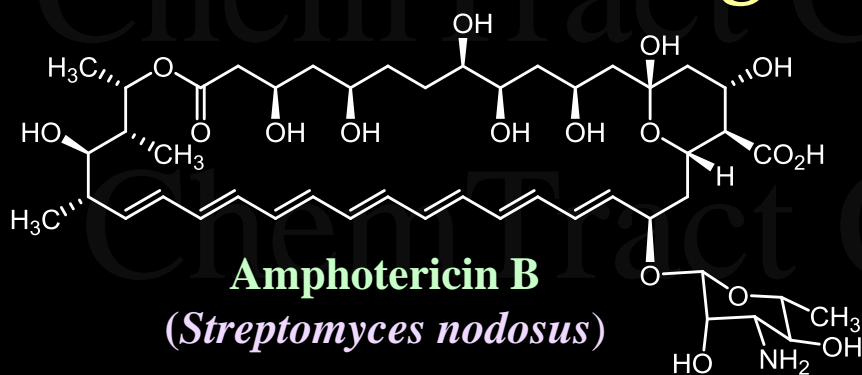
Drug Development Path



**Net Cost: \$802 Million
Invested Over 15 Years**

Source: Tufts Center for the Study of Drug Development

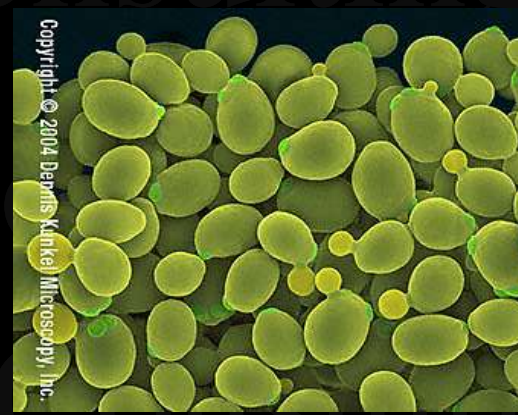
Antifungal Natural Products



Candida and *Aspergillus*: Most Prevalent Nosocomial Fungal Pathogens

- Yeasts (unicellular - buds)

- *Candida*
- *Cryptococcus*
- *Saccharomyces*



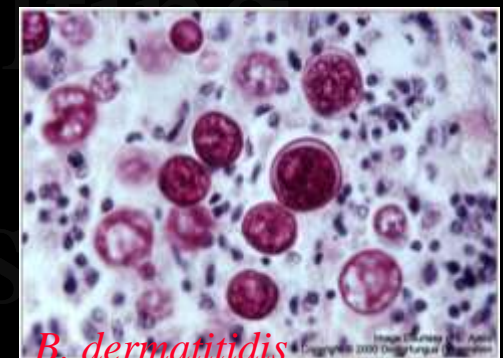
- Moulds (multicellular - hyphae and spores)

- *Aspergillus*
- *Fusarium*
- rare moulds
- *Trichophyton*



- Dimorphic (can grow in mould or yeast form)

- *Candida albicans*
- *Blastomyces dermatitidis*
- *Histoplasma capsulatum*



Localized Versus Disseminated Fungal Infection



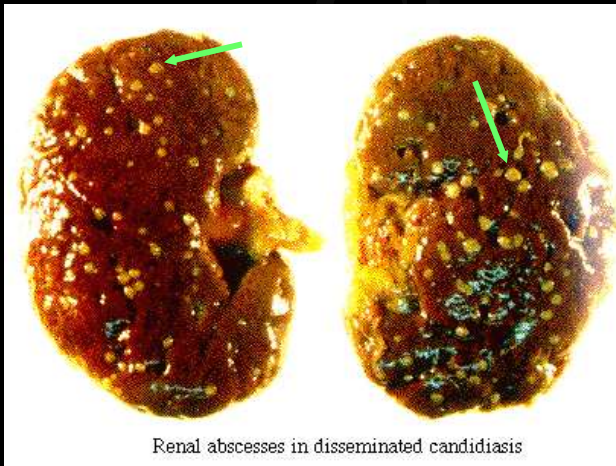
Candida albicans: oral thrush



Sporothrix schenckii: infected finger from rose thorn stick



Trichophyton mentagrophytes: tinea pedis (athlete's foot)



Renal abscesses in disseminated candidiasis

Candida albicans: disseminated infection showing kidney lesions

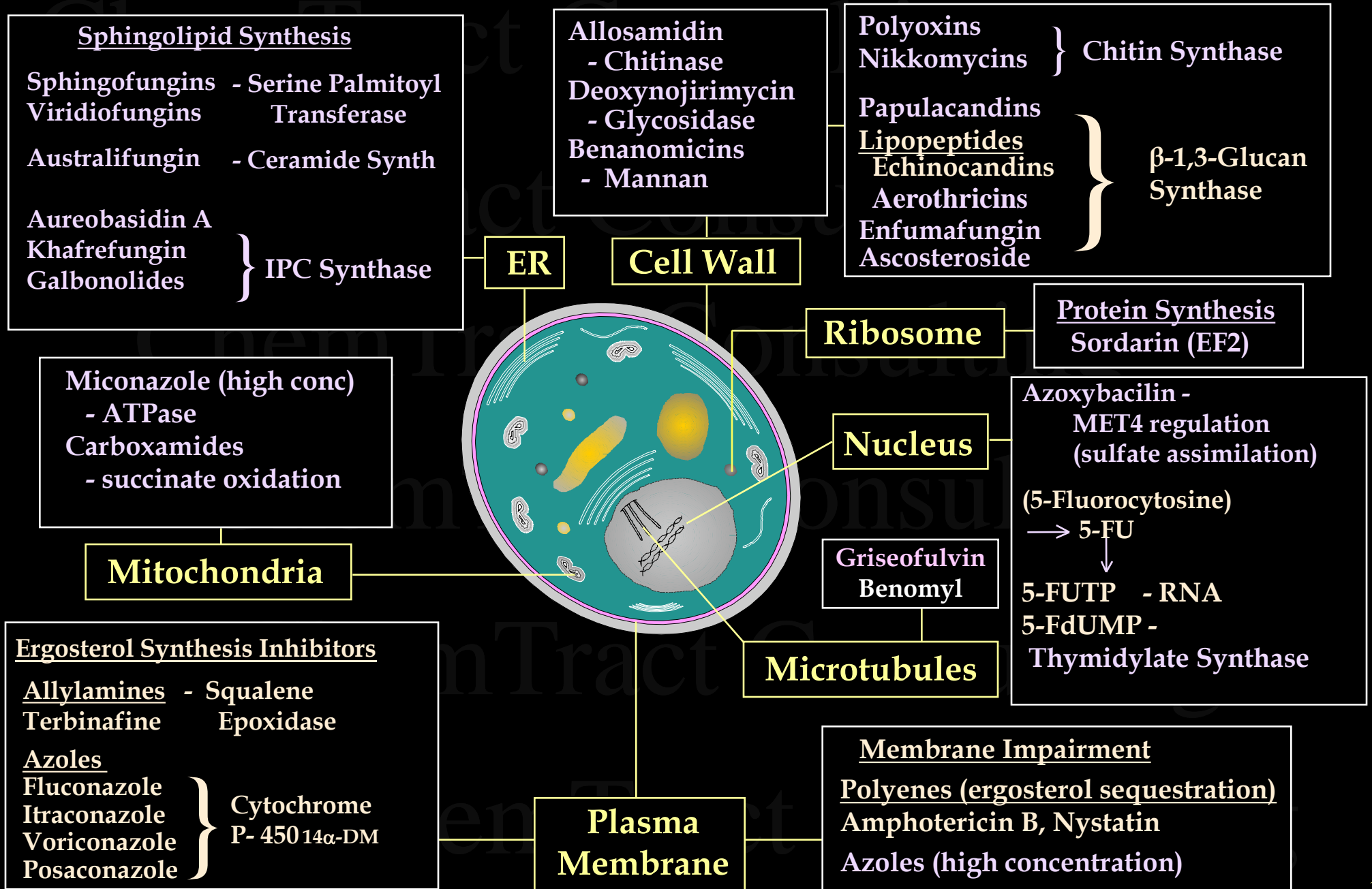


Aspergillus spp.: brain abscess

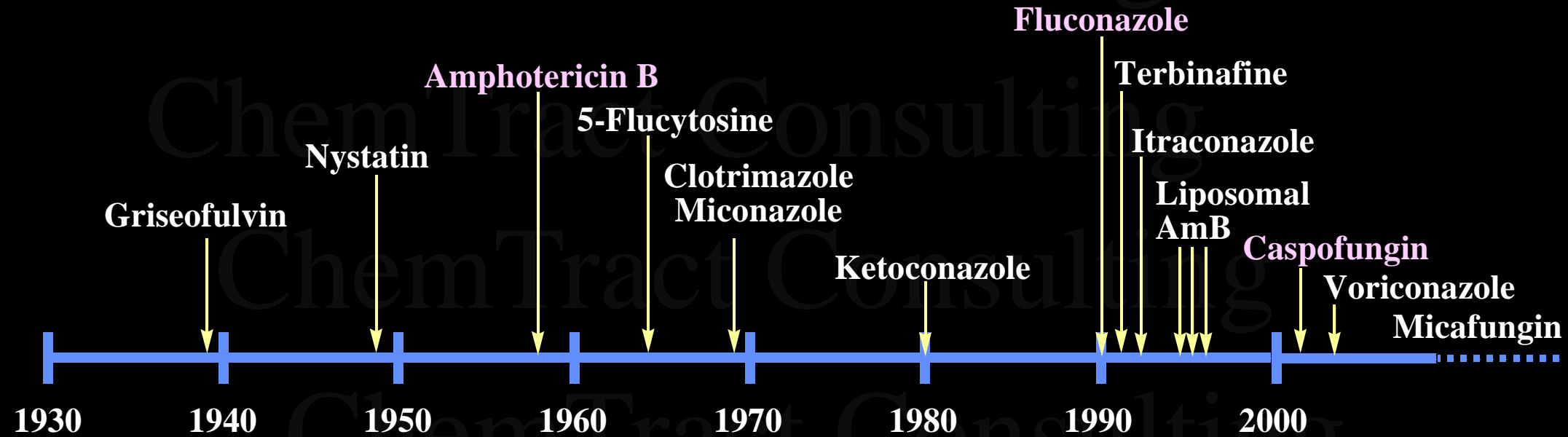


Aspergillus niger: fungal ball in lung, source of dis. infection

POTENTIAL TARGETS - SELECTIVITY IS KEY



Chronology of Antifungal Therapy



- Polyenes

- Pyrimidines

- Azoles

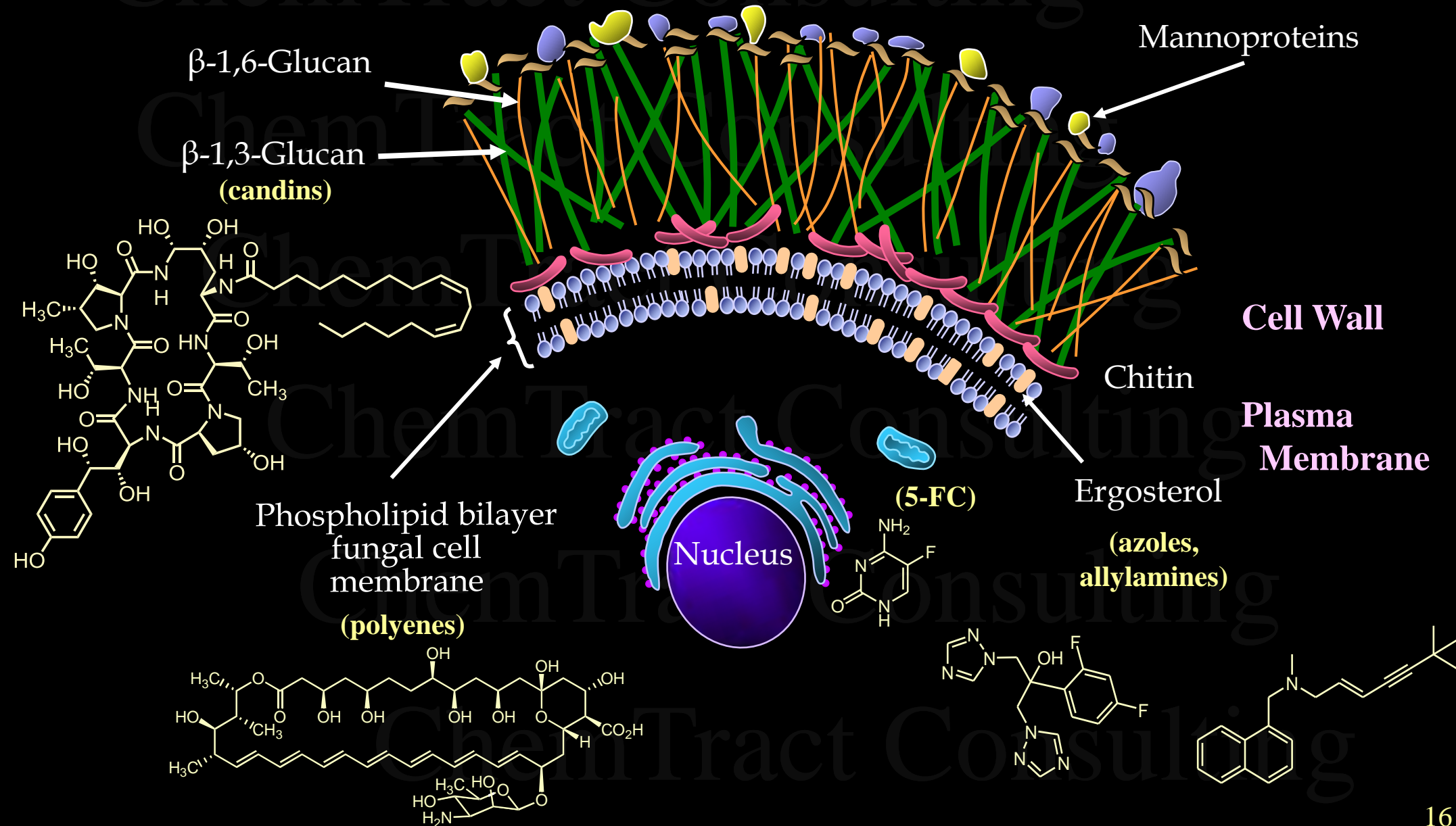
- Allylamines

- Echinocandins

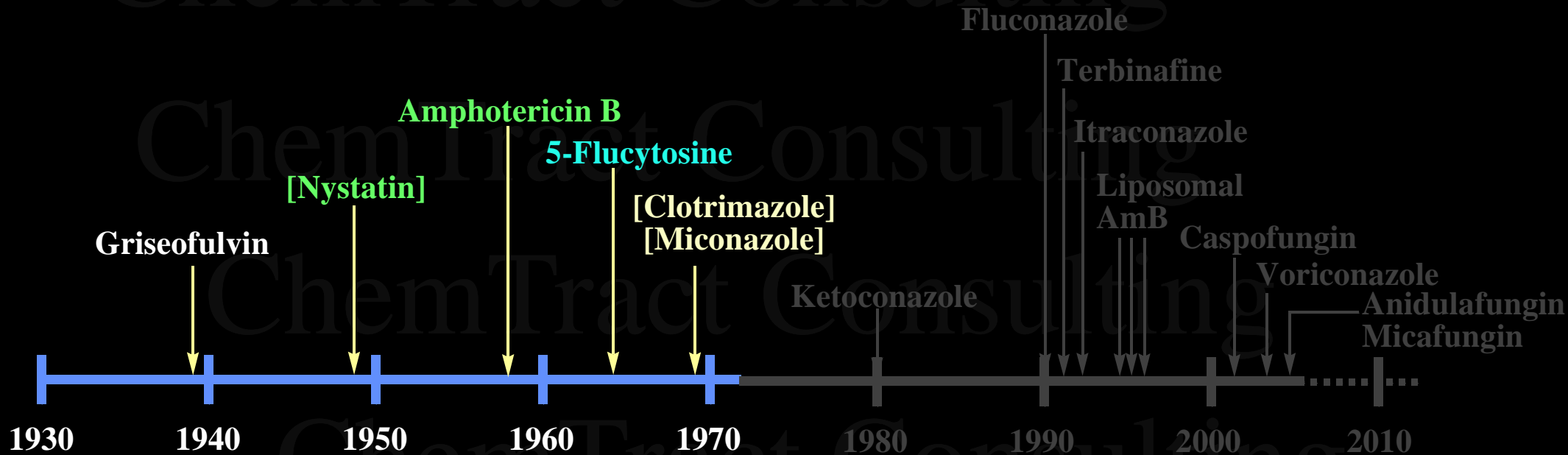
Baum *Postgrad. Med. J.* **55**, 587 (1978)

Andriole *Inf. Dis. Clin. Practice* **7** (supp 1), S2 (1998)

Systemically Administered Drugs and Targets



State of the Art Antifungal Therapy: 1970's

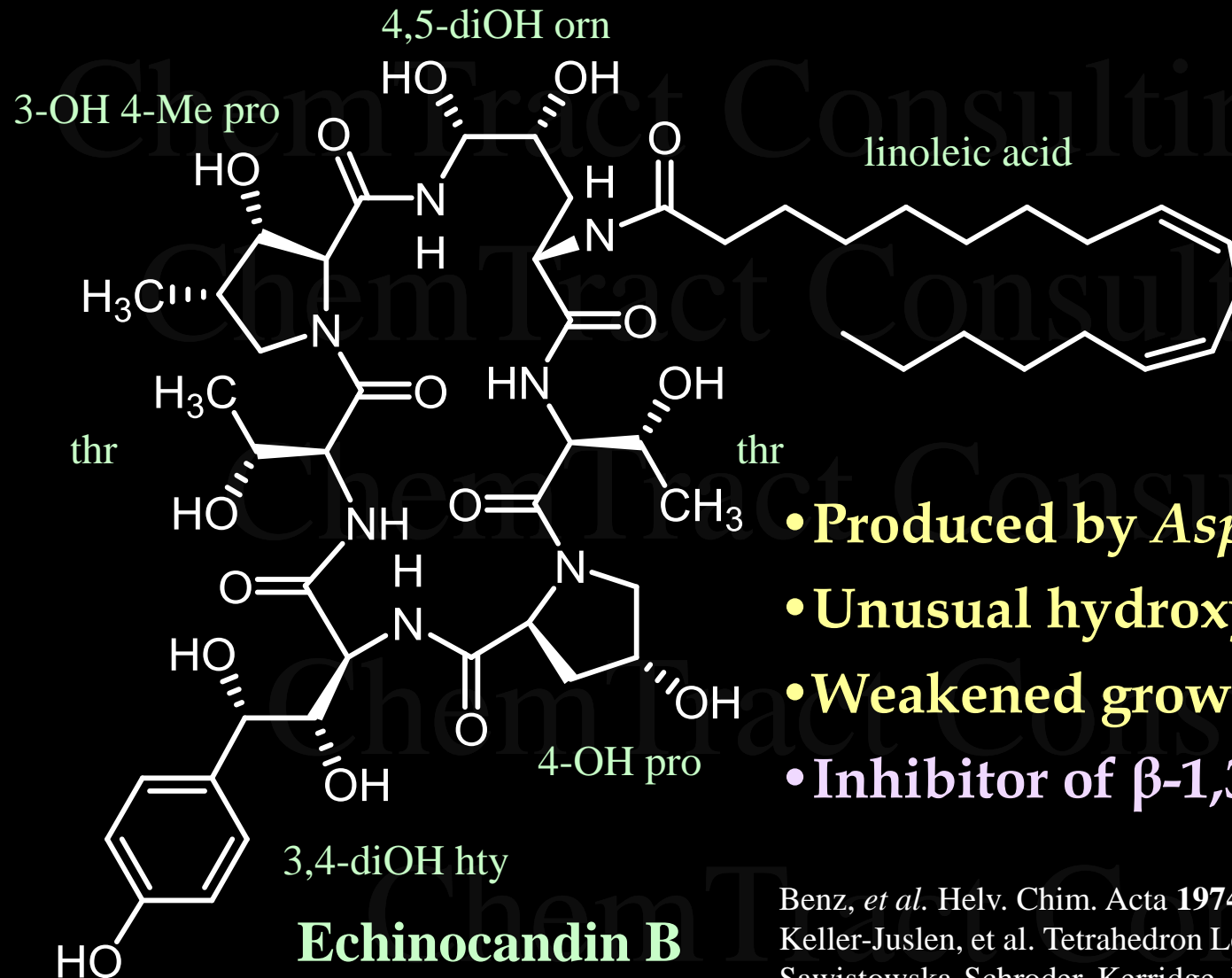


- Amphotericin B - broad spectrum, serious toxic effects
- 5-FC - rapid resistance development, limited spectrum

Baum *Postgrad. Med. J.* **55**, 587 (1978)

Andriole *Inf. Dis. Clin. Practice* **7** (supp 1), S2 (1998)

Early 1970's



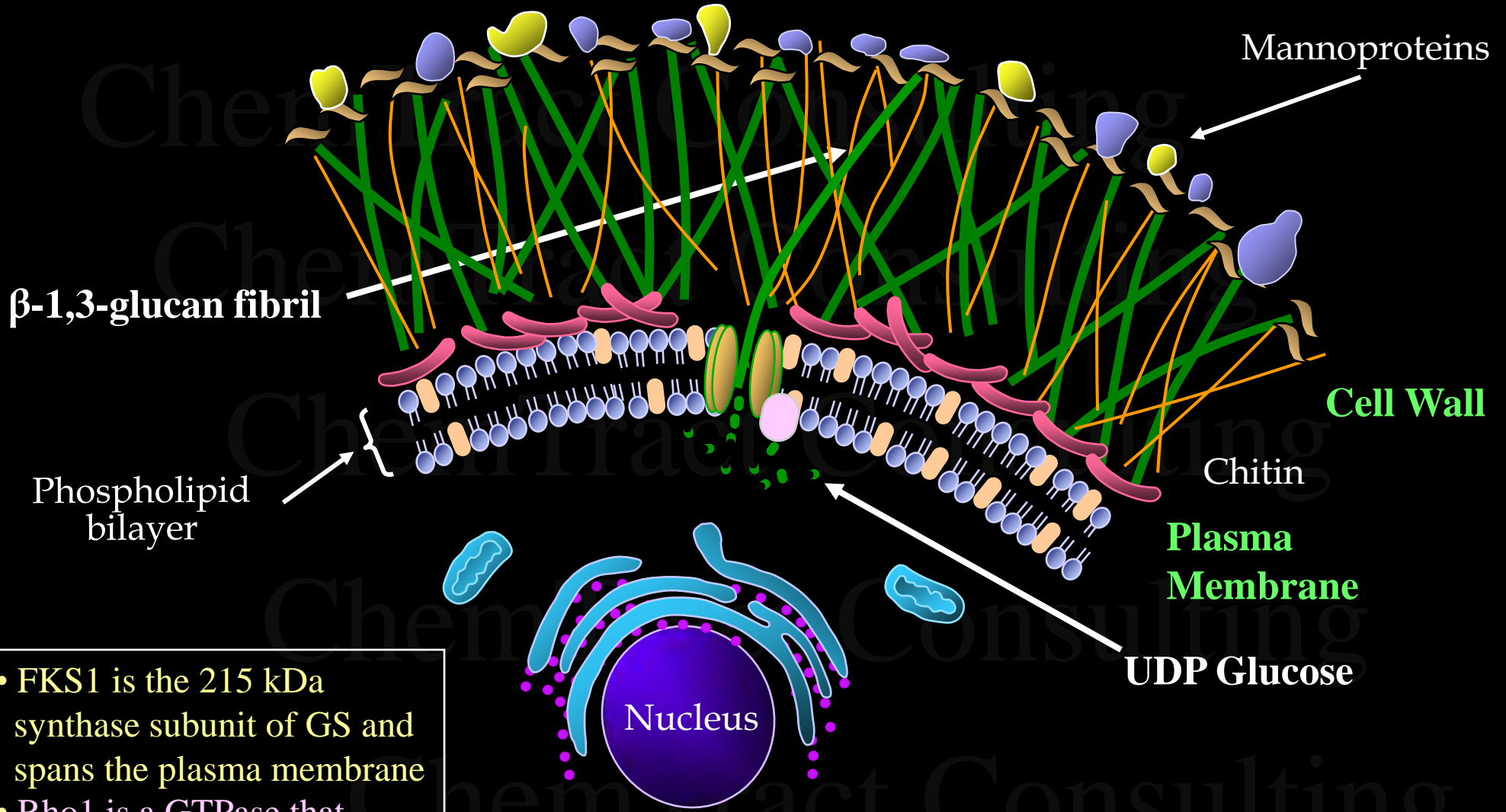
- Produced by *Aspergillus* spp.
- Unusual hydroxylated amino acids
- Weakened growing fungal cell wall
- Inhibitor of β -1,3-glucan synthesis

Benz, *et al.* Helv. Chim. Acta **1974**, 57: 2459-77

Keller-Juslen, *et al.* Tetrahedron Lett. **1976**, 4147-50

Sawistowska-Schroder, Kerridge, Perry, FEBS Lett. **1984**, 173: 134-138

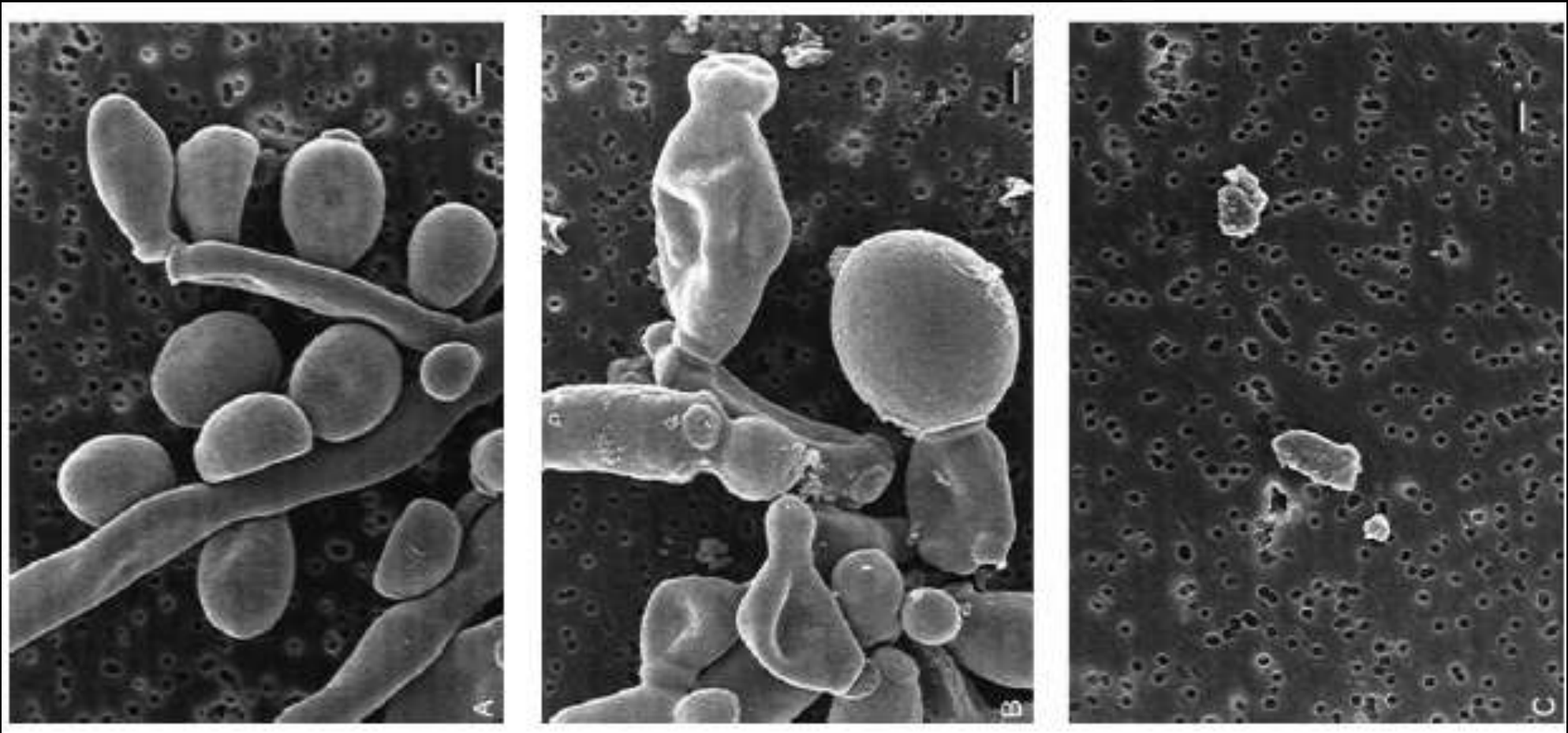
β -1,3-Glucan Synthesis In Fungi



- FKS1 is the 215 kDa synthase subunit of GS and spans the plasma membrane
- Rho1 is a GTPase that upregulates glucan synthesis

spatial and temporal orchestration of cell structure

Morphological Effect of GS Inhibitor (MK-0991) on *C. albicans*



no inhibitor

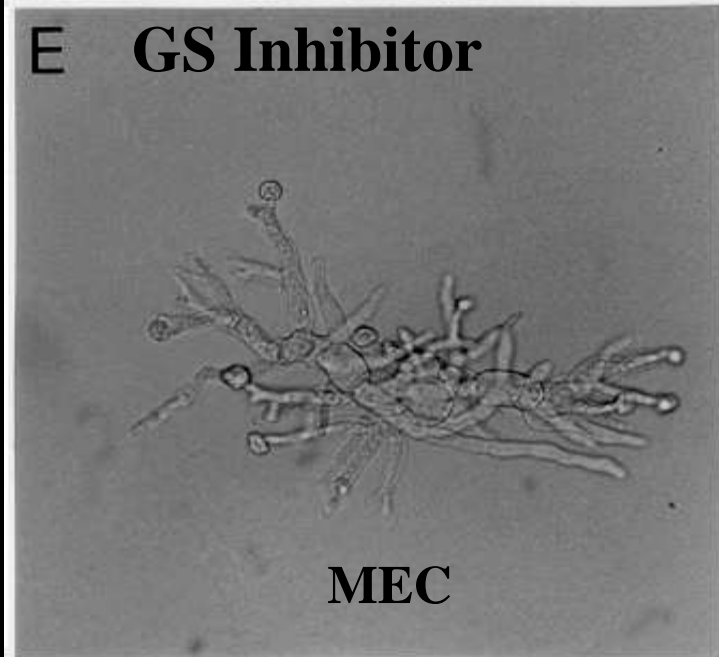
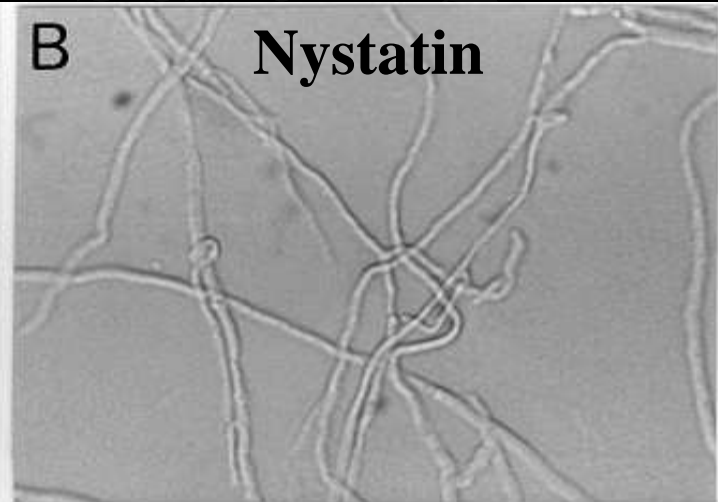
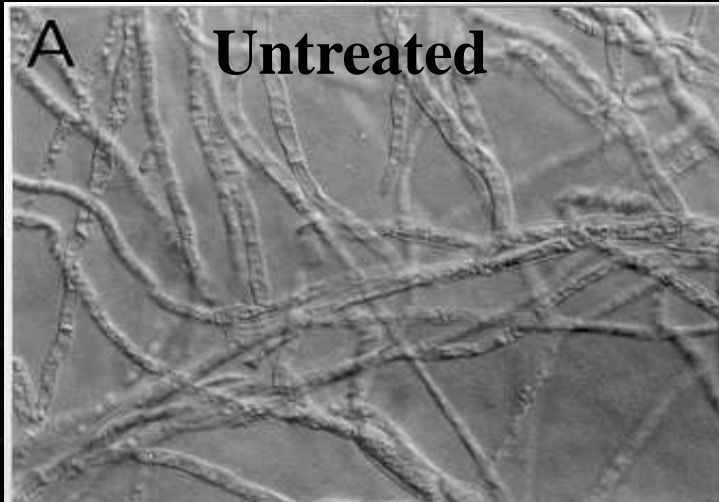
MIC₈₀

MIC₁₀₀

[| = 1 μ m]

E. Ernst, *et al.* Diagn Microbiol. Infect. Dis. 1999, 33: 75-80

Aspergillus Morphology



Lozoya Valley, Spain 1985



- sample was collected and first identified by CIBE laboratory in Madrid, Spain

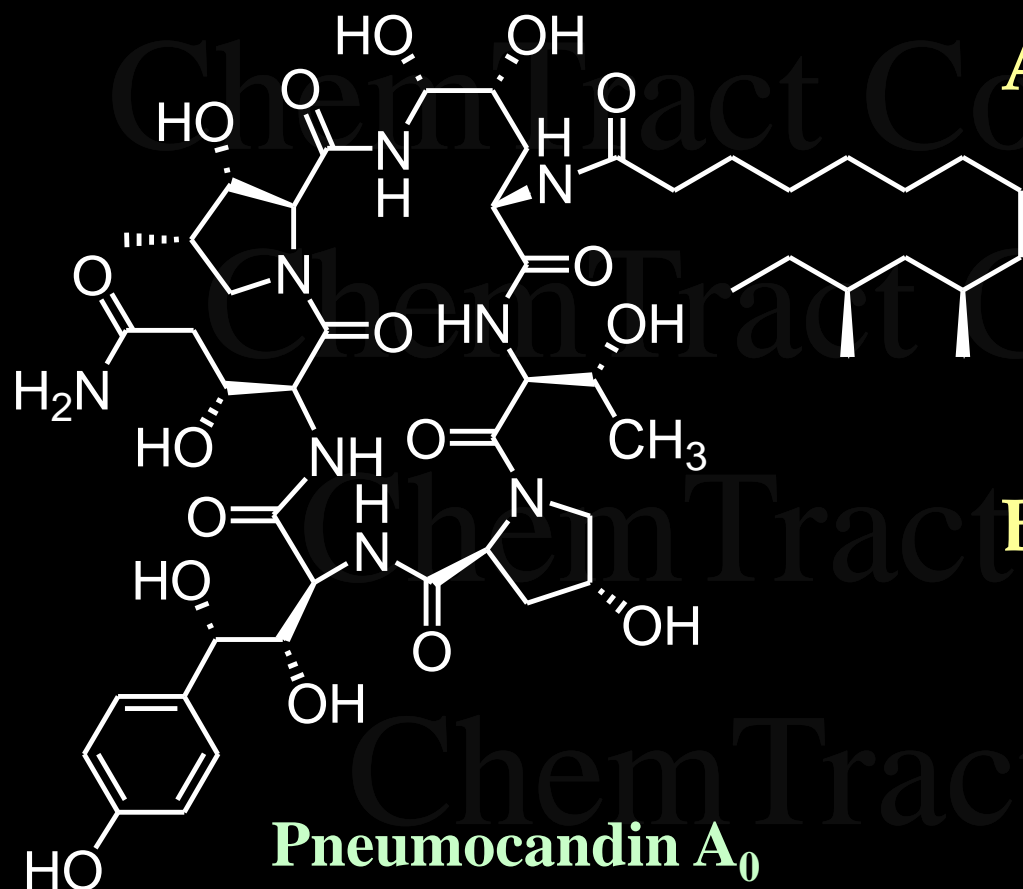
Glarea lozoyensis



originally characterized
as *Zalerion arboricola*

Bills *et al.*, Mycol. Res. 1999, 103: 179-192

1987



A Great Lead

- fungicidal and novel mechanism of action
- active in vivo
- few off-target activities

But...

- spectrum limited to several *Candida* spp.
- not orally bioavailable
- not water soluble

Schwartz *et al.* J. Antibiot. 1992, 45: 1853-66
Leonard *et al.* Org. Letters, 2002, 4: 4201-04

1989

The Peak of the AIDS Epidemic

#1 Infection:

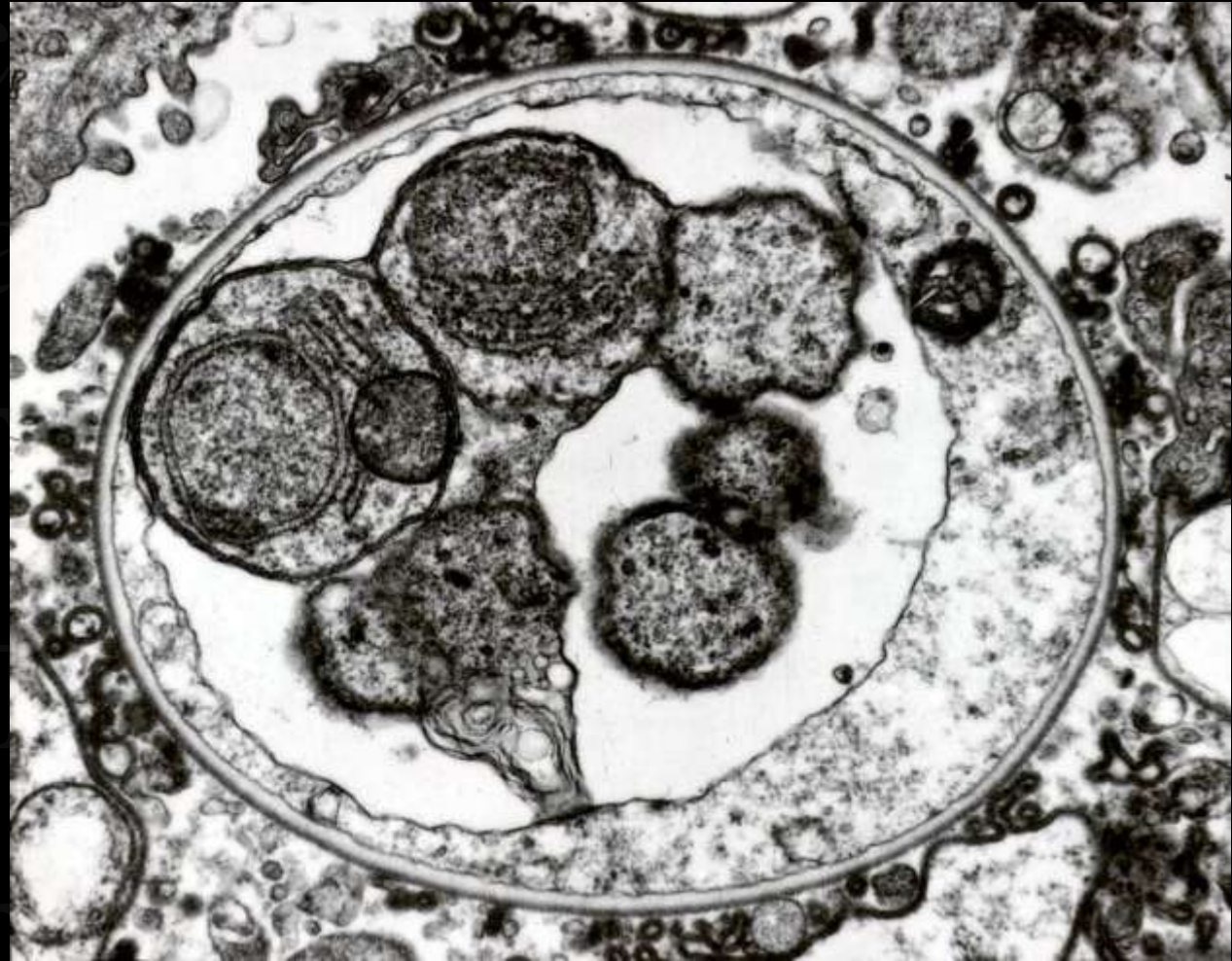
Oral Thrush from *Candida*

#1 Cause of Death:

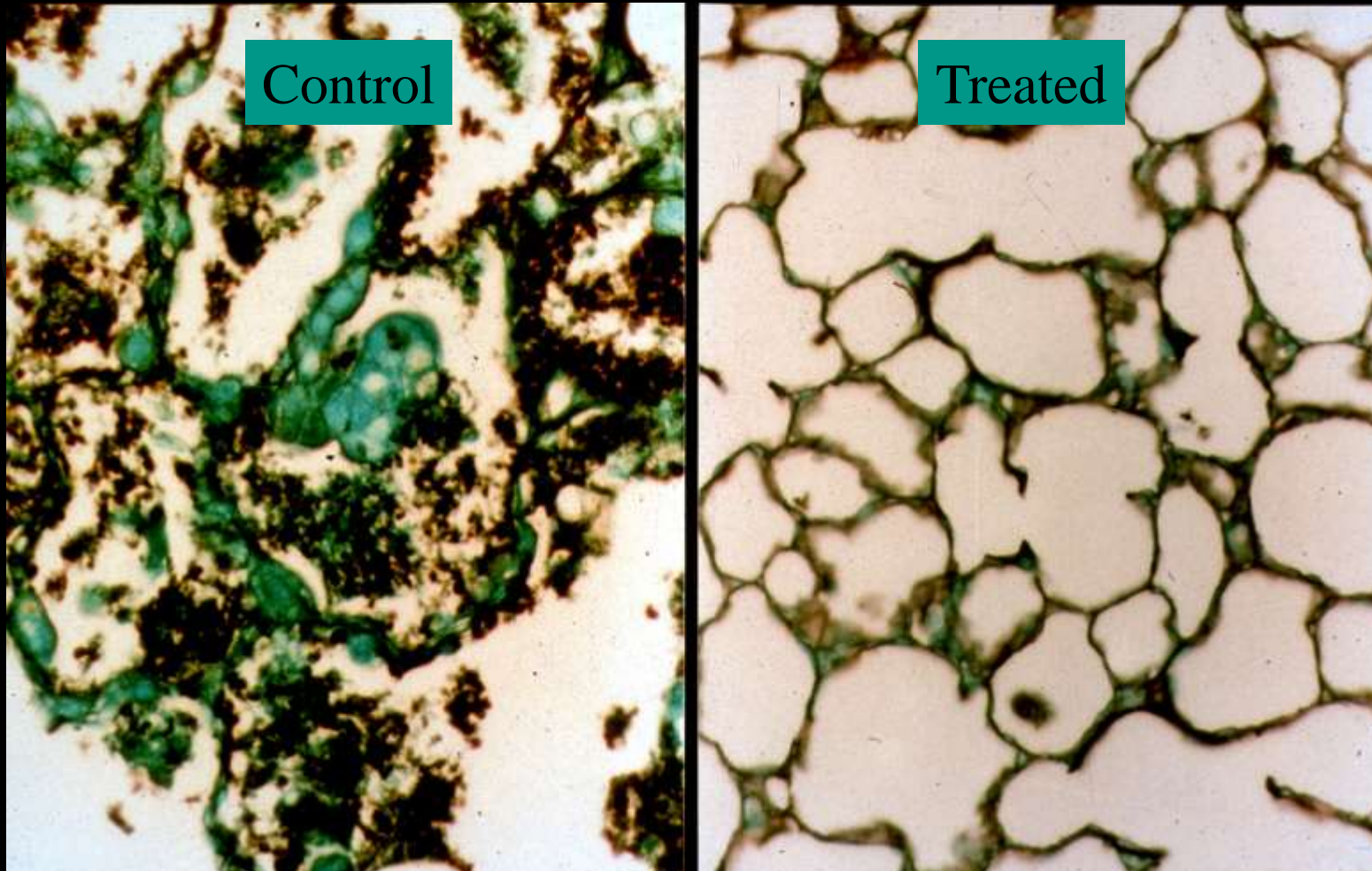
Pneumocystis Pneumonia

Pneumocystis carinii (*P. jiroveci* in humans) Cyst

β -1,3-D-glucan



Pneumocandin Prevented Pneumocystis Pneumonia In Immune Compromised Rats



alveoli of lung

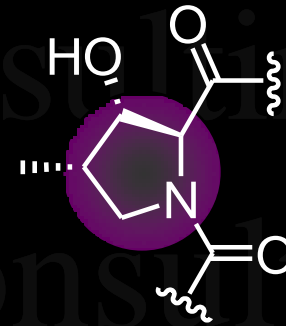
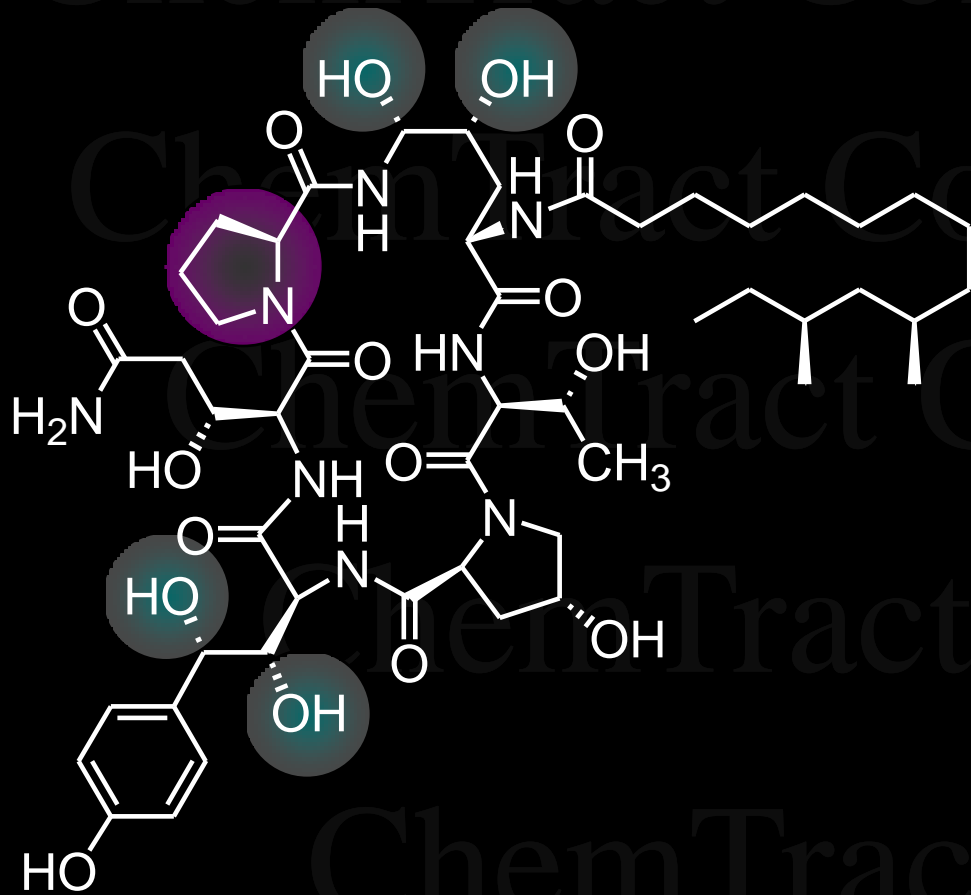
Schmatz, et al. *J. Protozoology* **1991**, 38 (6): S151-S153

Powles, et al. *Antimicrob. Agents Chemother.* **1994**, 38 (6): 1397-1401

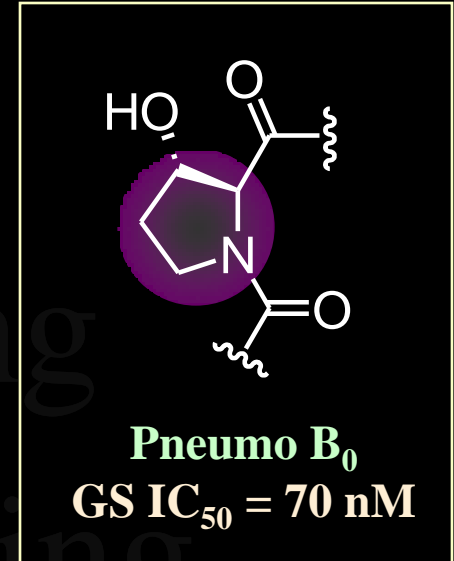
Potential Therapy For AIDS Patients

- Single agent for the most common infections in AIDS patients
 - oral thrush from *Candida albicans* (and others)
 - *Pneumocystis pneumonia*
- Medicinal Chemistry program initiated at Merck's Rahway, NJ laboratories in 1989

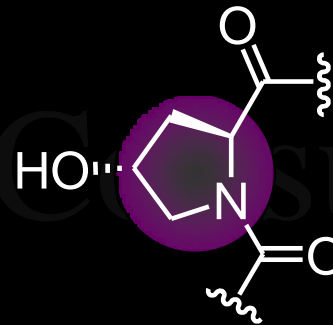
The Pneumocandins



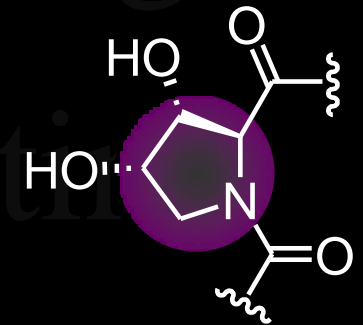
Pneumo A₀
GS IC₅₀ = 200 nM



Pneumo B₀
GS IC₅₀ = 70 nM



Pneumo C₀
GS IC₅₀ = 500 nM



Pneumo D₀
GS IC₅₀ = 120 nM

Masurekar, *et al.* J. Antibiot. **1992**, 45: 1867-74

Hensens, *et al.* *ibid* **1992**, 45: 1875-85

Morris, *et al.* J. Antibiot. **1994**, 47: 755-764

Petersen, *et al.* J. Ind. Microbiol. Biotechnol. **2001**, 26: 216-21

In Vitro Assays

- **GS IC₅₀**
 - inhibition of β -1,3-glucan synthesis in *Candida albicans* semi-purified membranes. Can be determined for any *Candida* or *Aspergillus* spp.
- **MFC/MEC**
 - minimum fungicidal concentration: broth microdilution assay against a panel of yeast and filamentous fungi. **MEC** is the minimum concentration effecting a morphological change in filamentous fungi.

In Vivo Models

- **TOKA (Target Organ Kidney Assay)**
 - mouse candidiasis tissue burden model
- **ASP**
 - mouse aspergillosis survival model

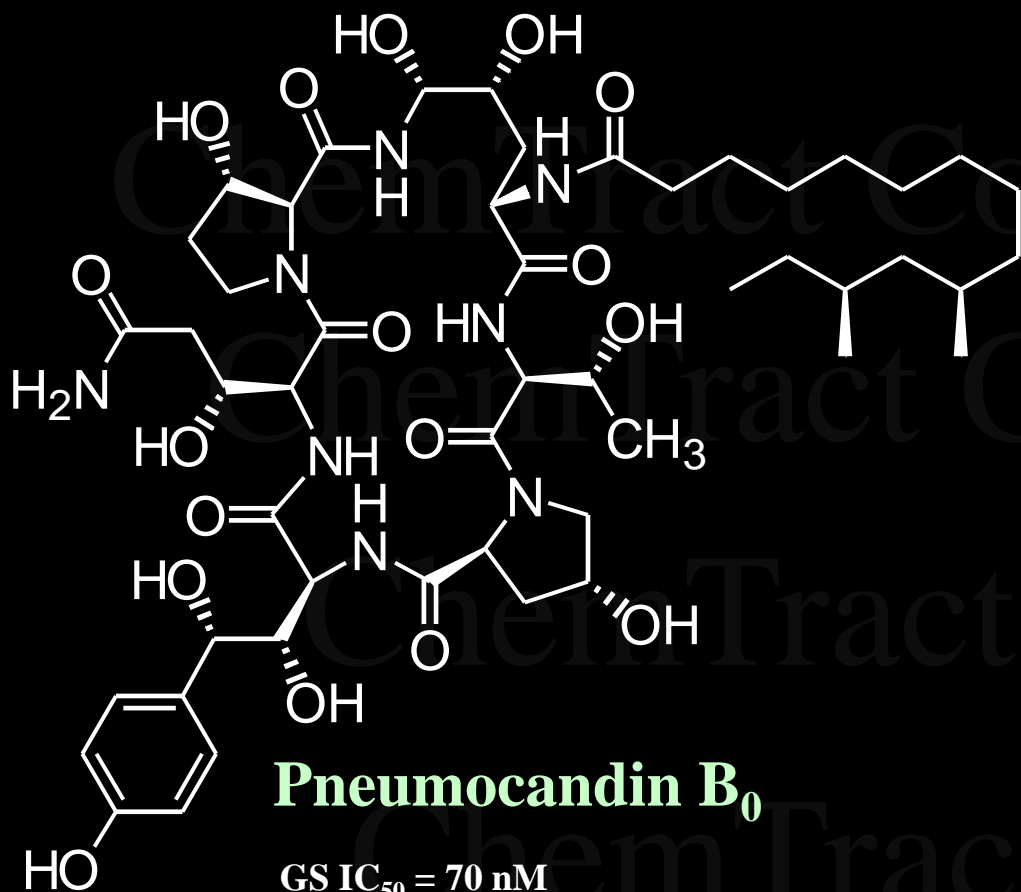
Disseminated Candidiasis Model (TOKA)



Disseminated Aspergillosis Model (ASP)



Medicinal Chemistry Lead - 1989



GS IC₅₀ = 70 nM

MFC/MEC (µg/mL)

C. alb, *C. parap*, *A. fum* = 0.25, 4, 1*

In Vivo Activity in Mice

99.9% redn CFUs in kidneys @ 6 mg/kg (*C. alb*)
inactive in disseminated aspergillosis model

A Great Lead...

- fungicidal
- novel mechanism of action
- active in mouse candidiasis model
- few off-target activities
- novel (patentable) structure

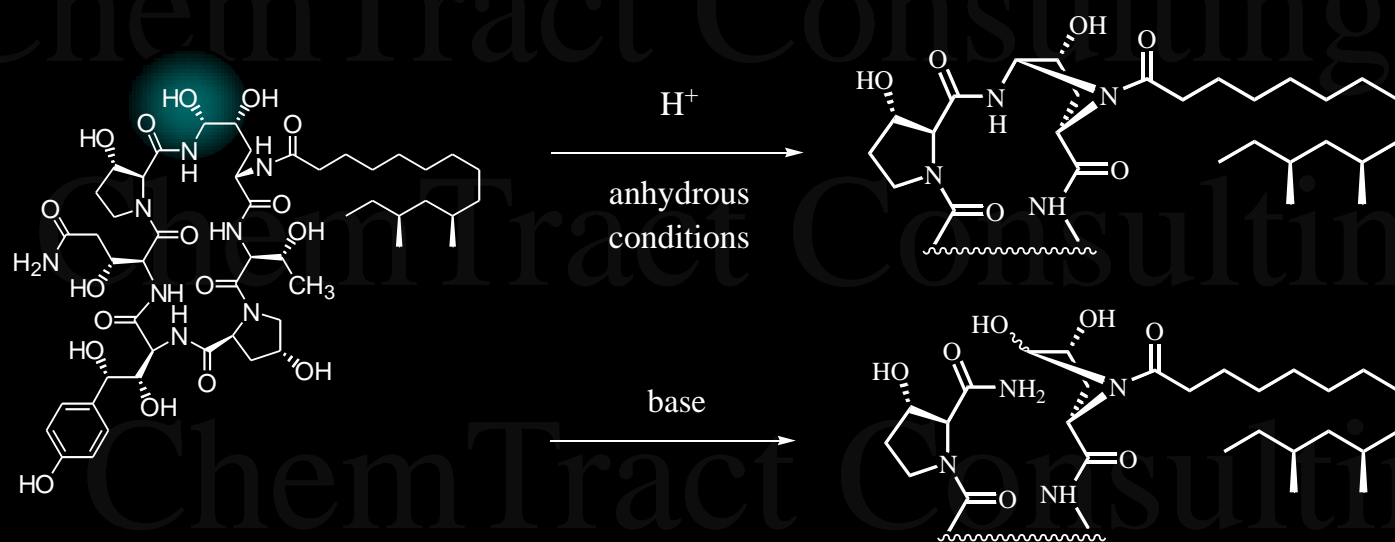
With Some Limitations...

- higher MICs against some important *Candida* spp.
- PK - fast Clp and short t_{1/2}
- low oral bioavailability (<1%)
- poorly water soluble (<0.1 mg/mL)

Schwartz *et al.* J. Antibiot. 1992, 45: 1853-66
Leonard *et al.* Org. Letters, 2002, 4: 4201-04

Initial Program Goals

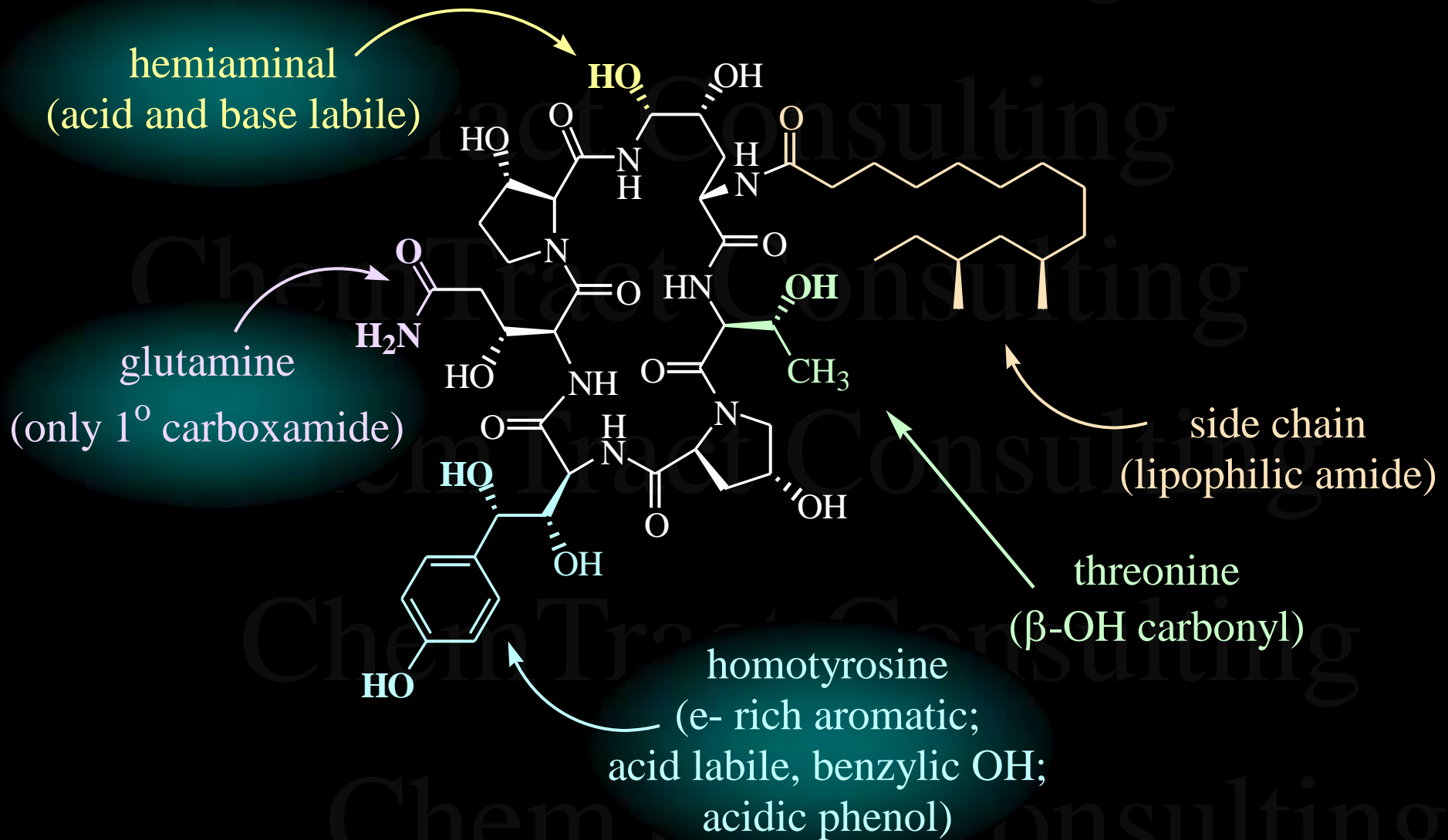
- Water solubility to allow I.V. formulation development
- Increase potency (expand spectrum?)
- Improve chemical stability



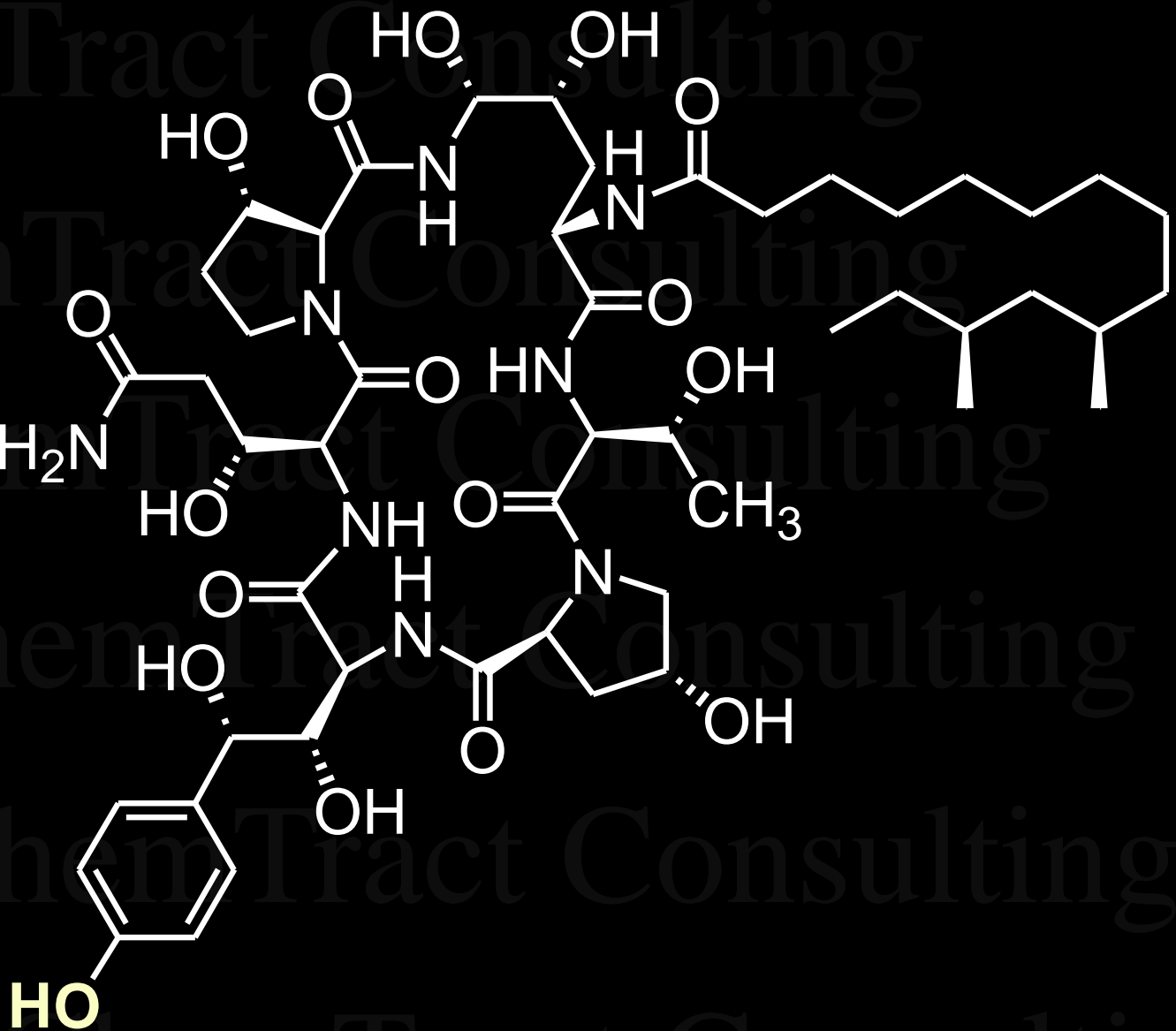
Bouffard, et al. *Tetrahedron Lett.* **36**, 1405 (1995)

- Pharmacokinetics to minimally support bid dosing

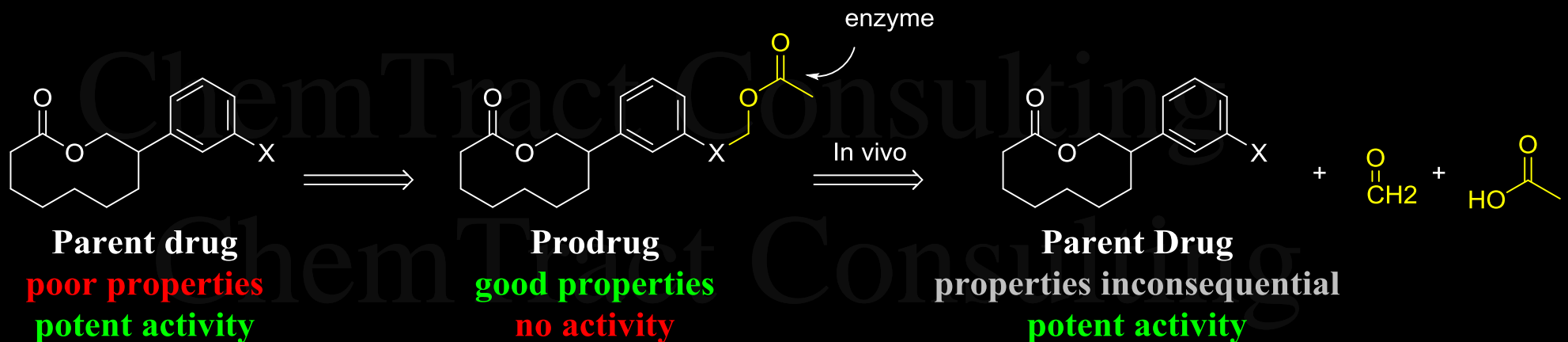
Chemical Modifications (1989-1995)



phenol

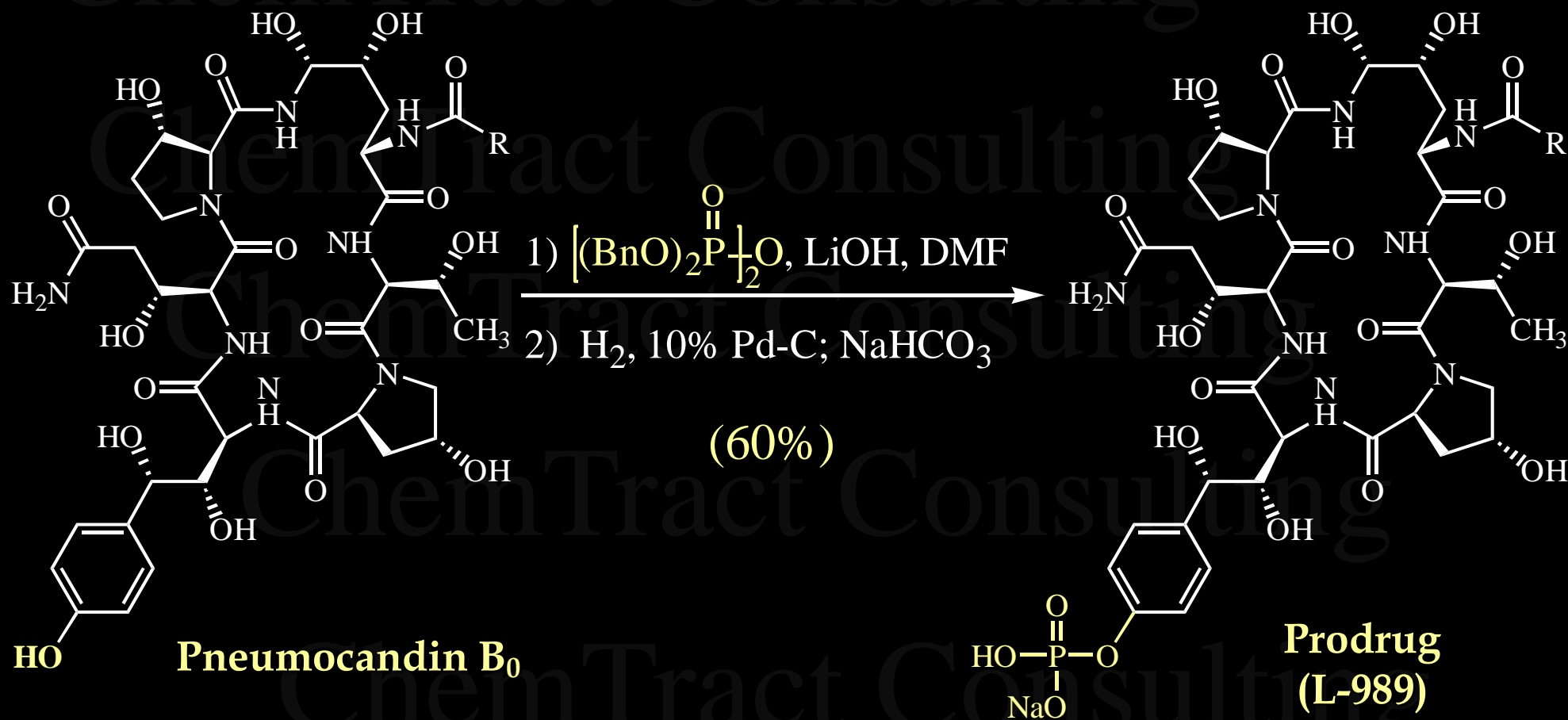


Prodrugs



- Parent drug lacks some important property (solubility, cell penetration, oral bioavailability)
- Modification imparts the desired property and ideally the resulting prodrug would be inactive itself
- Group that imparted the desired property is readily cleaved when needed to release active parent drug

Phenol Prodrug Derivatives



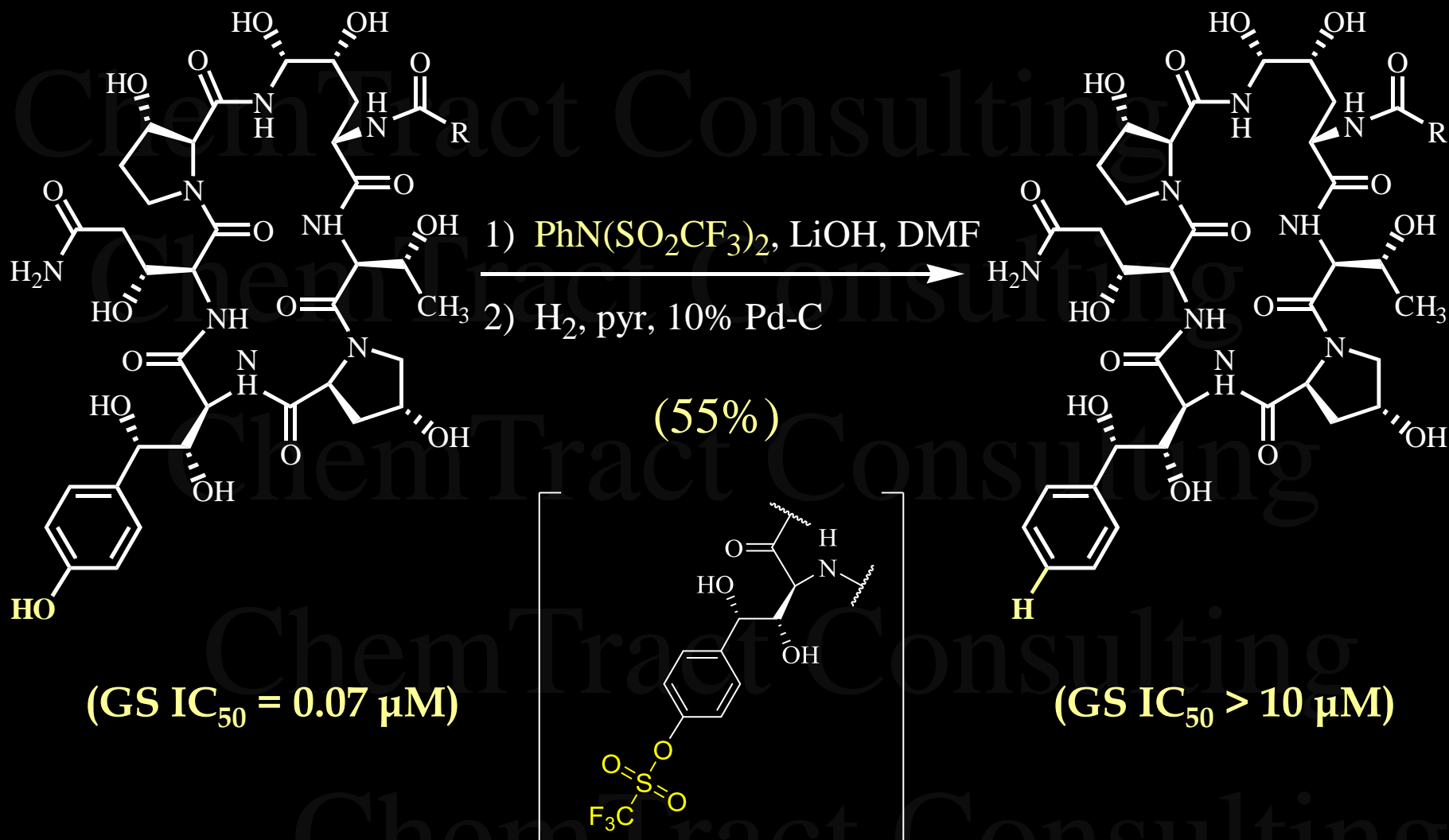
A series of carboxylate, carbamate, carbonate and phosphate esters bearing a charged (+ or -) group were prepared and evaluated for solubility and chemical and biological stability

J. Med. Chem. 35, 194 (1992)

In Vitro Antifungal Activity

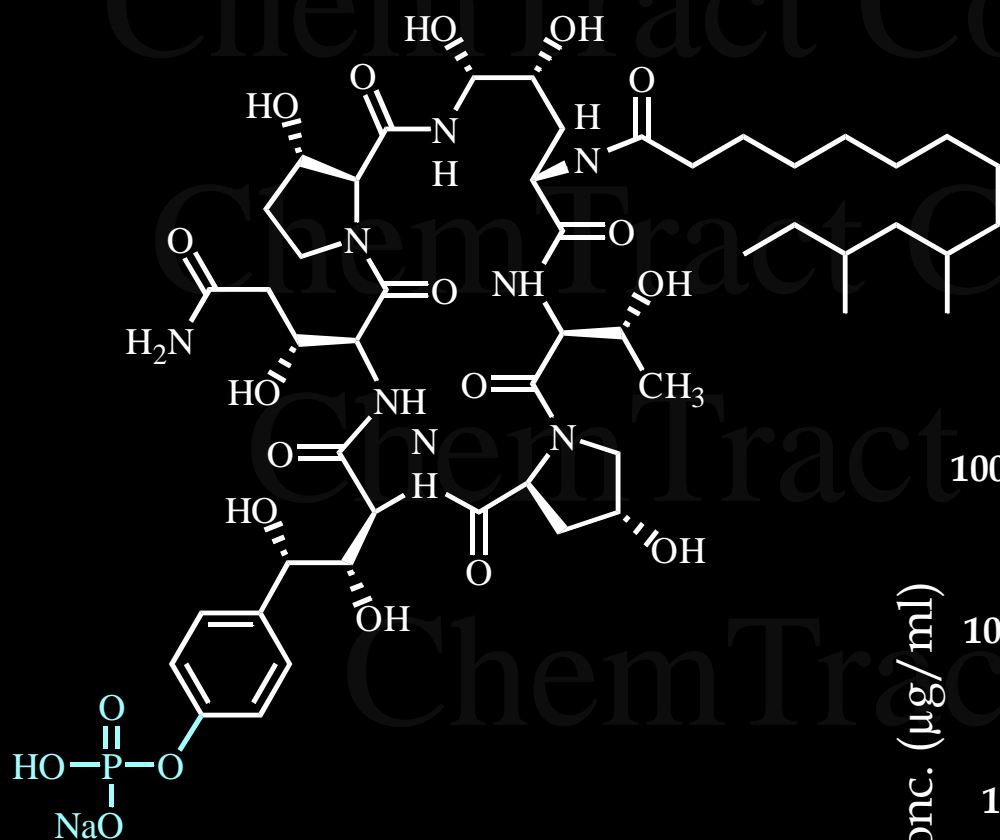
<u>Assay</u>	<u>L-989</u>	<u>Pneumo B₀</u>
Sol (mg/mL)	>50	<0.1
GS IC₅₀ (μM)	>10	0.07
MFC (μg/mL)		
<i>C. albicans</i>	4	0.25
<i>C. parapsilosis</i>	32	4

Phenol Is Important For Activity



Bioorg. Med. Chem. Lett. 3 2039 (1993)

First Development Candidate



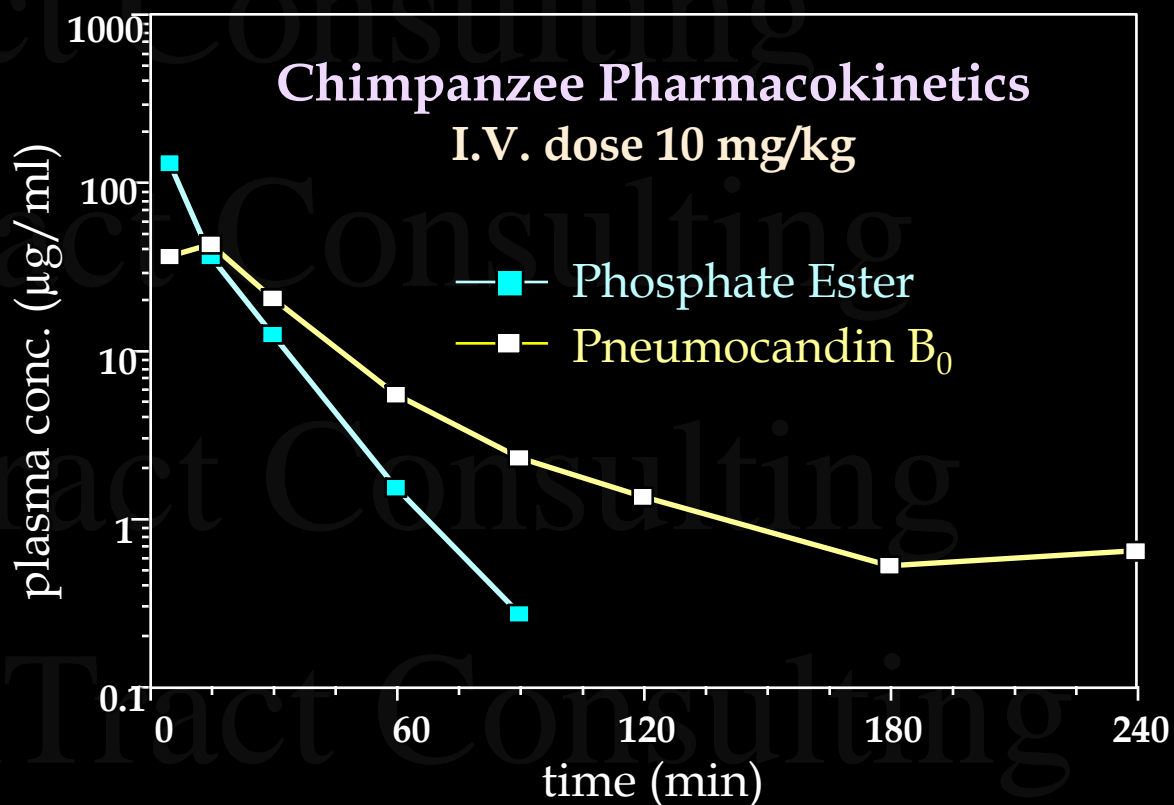
- Water soluble (>50 mg/mL)
- Chemically stable ($t_{1/2} \gg 100$ h)
- Equivalent potency to natural product *in vivo* (still lacked *Aspergillus* activity)

GS IC_{50} (nM): >10,000 (70 nM for parent)

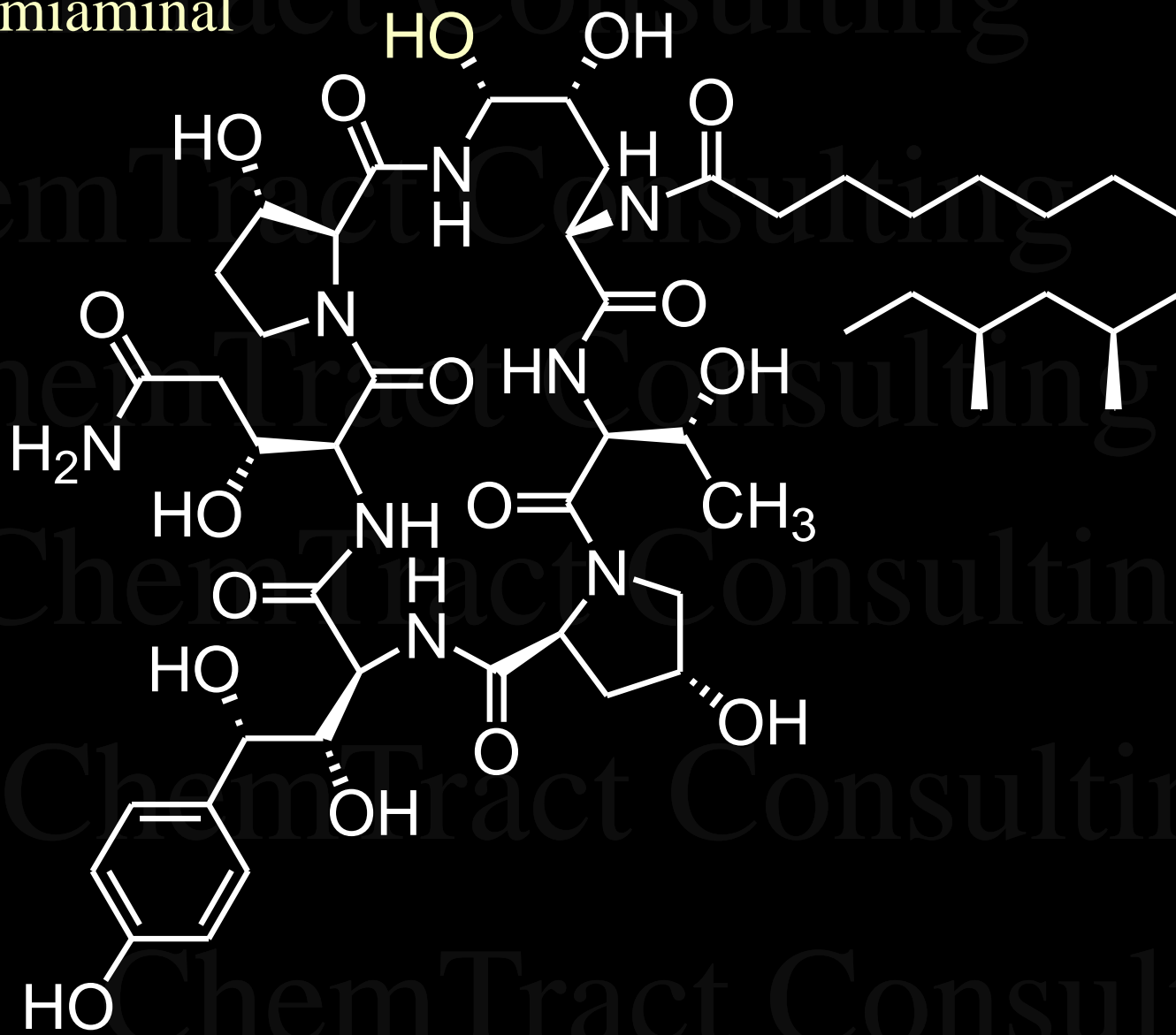
(60% yield in 2 steps using tetrabenzylpyrophosphate)

J. Med. Chem. **1992**, 35: 194-196

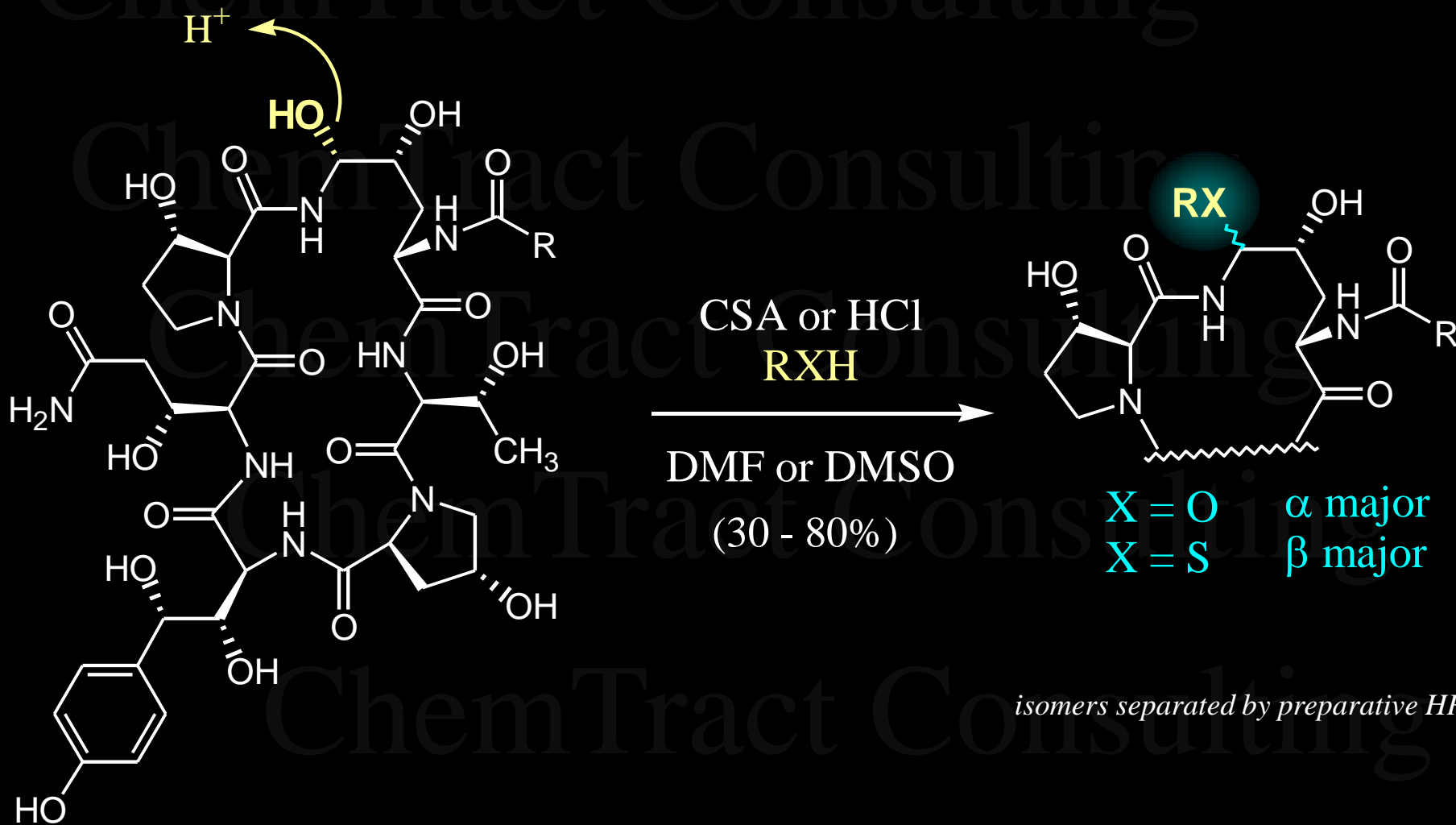
Bioorg. Med. Chem. Lett. **1993**, 3: 2039-42



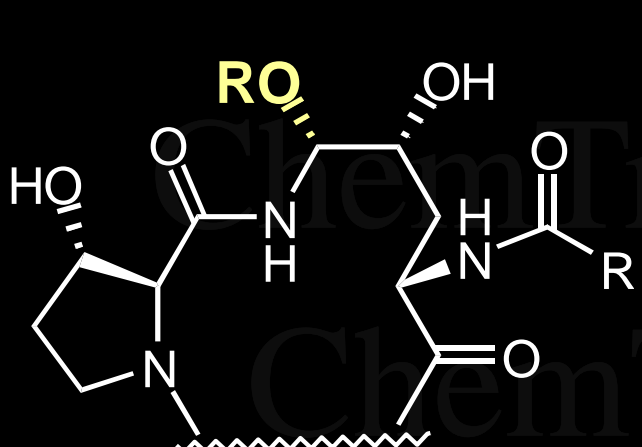
hemiaminal



C5-Orn Substitution

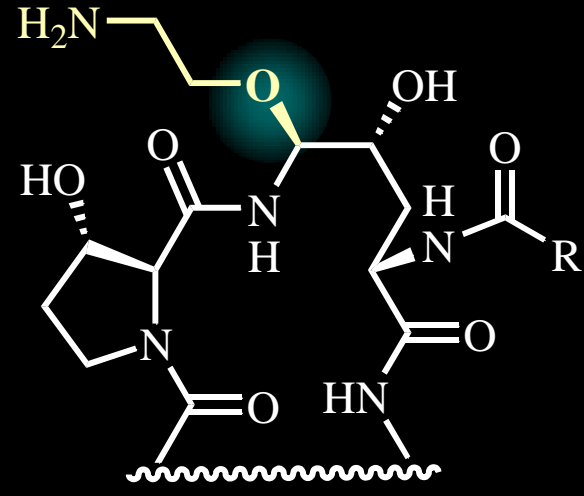
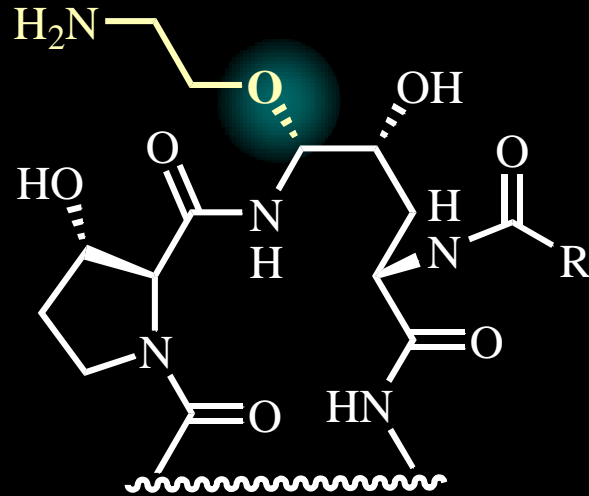


Cationic Ethers Improve Activity and Spectrum



	-OH	-OCH ₃	-OCH ₂ CH ₃	-OCH ₂ CH ₂ OH	-OCH ₂ CH ₂ NH ₂	
GS IC₅₀	70	100	800	400	10	(nM)
MFC						
<i>C. albicans</i>	0.25	2	4	2	0.125	(μg/mL)
TOKA (ED₉₉)	3	>6	-	-	0.3	(mg/kg)
ASP (ED₅₀)	>20	1.8	-	-	0.06	(mg/kg)

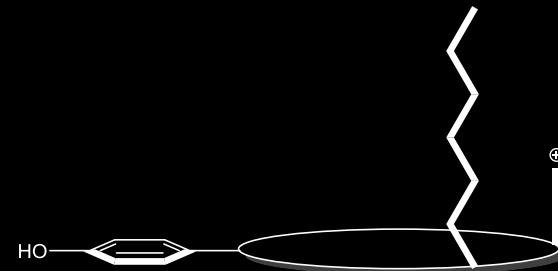
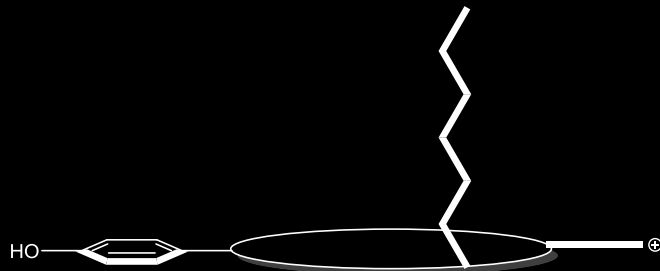
Activity of Epimeric Pair



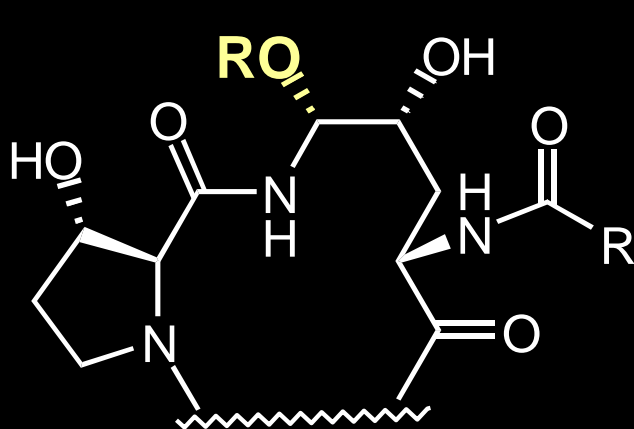
GS IC₅₀ (nM)

10

450

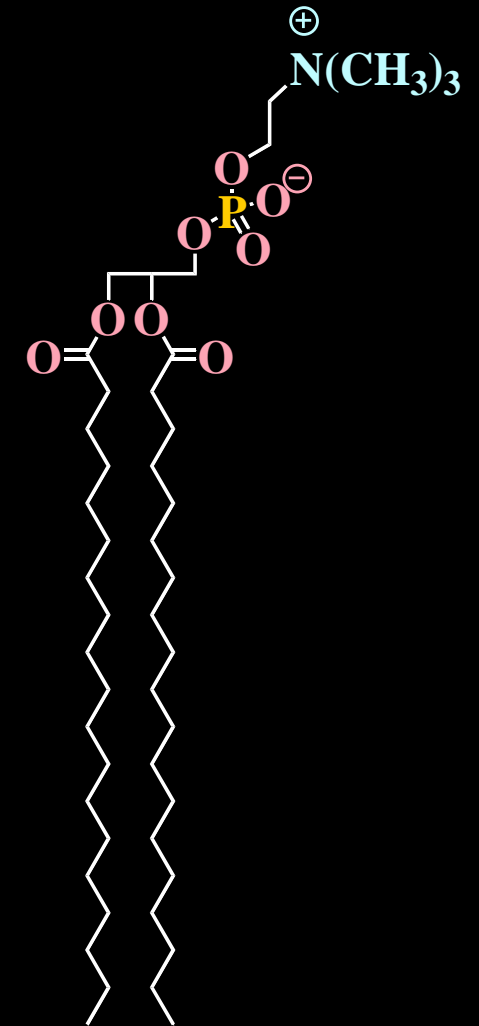
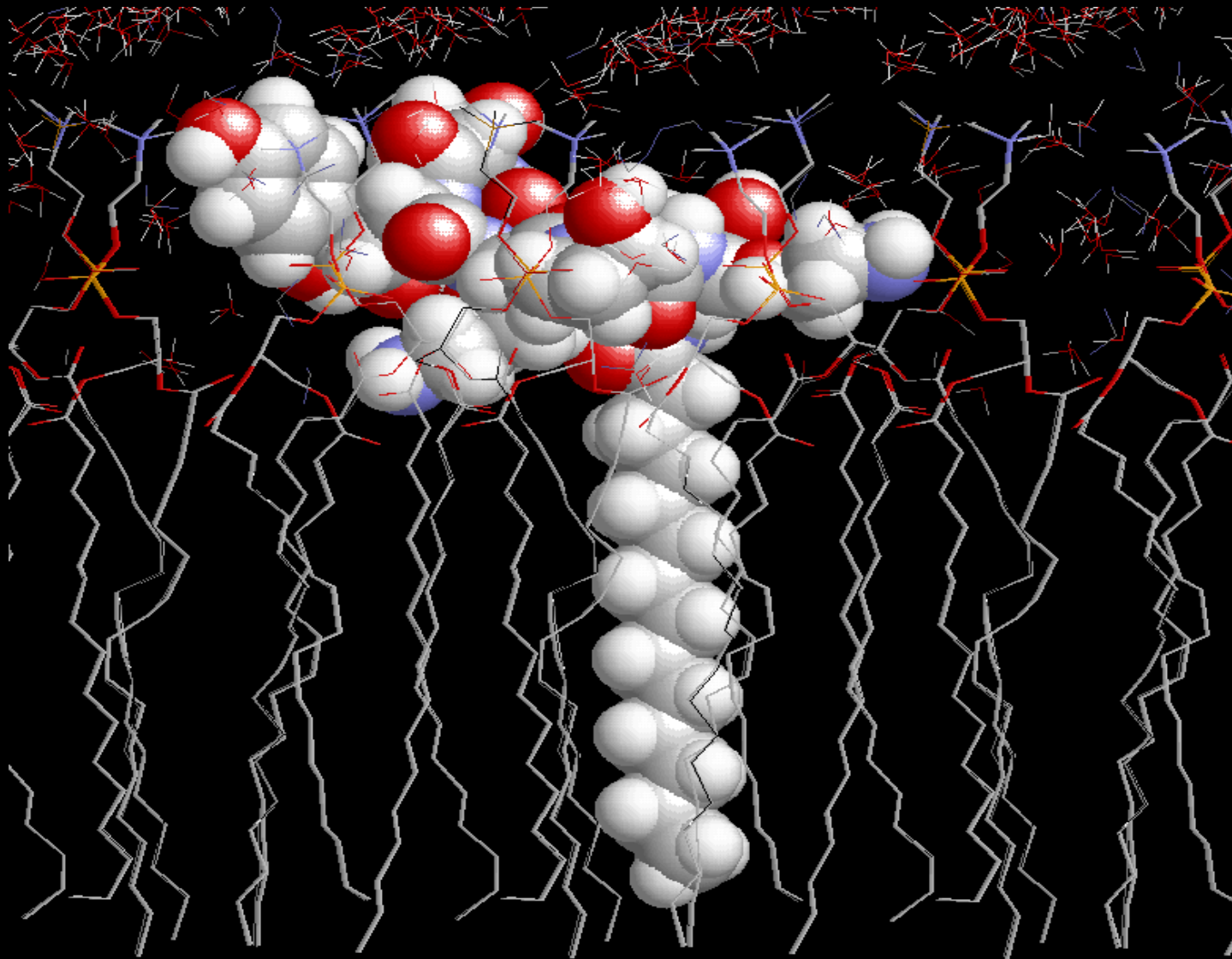


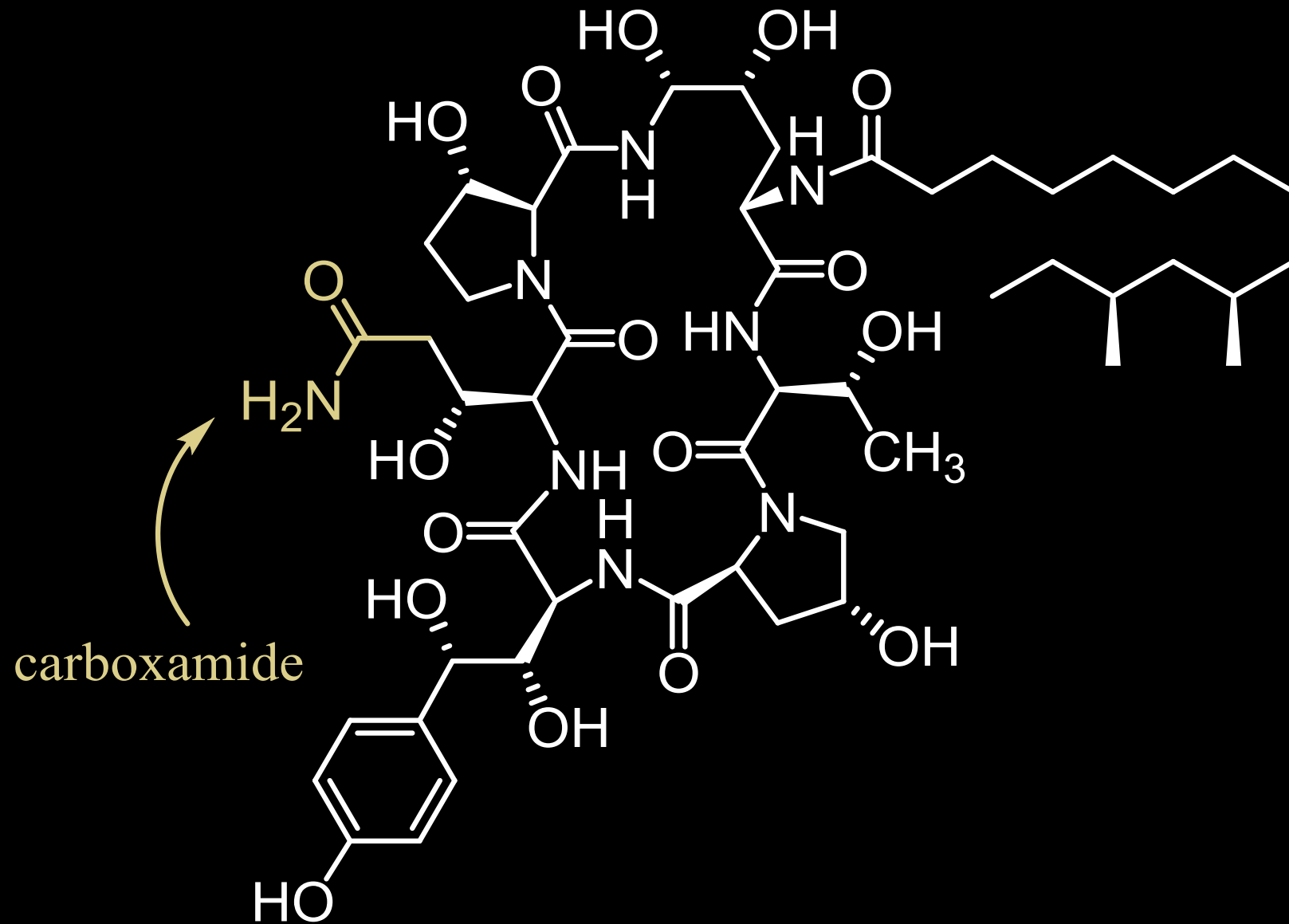
Tether Length Had Only Minor Effect on Activity



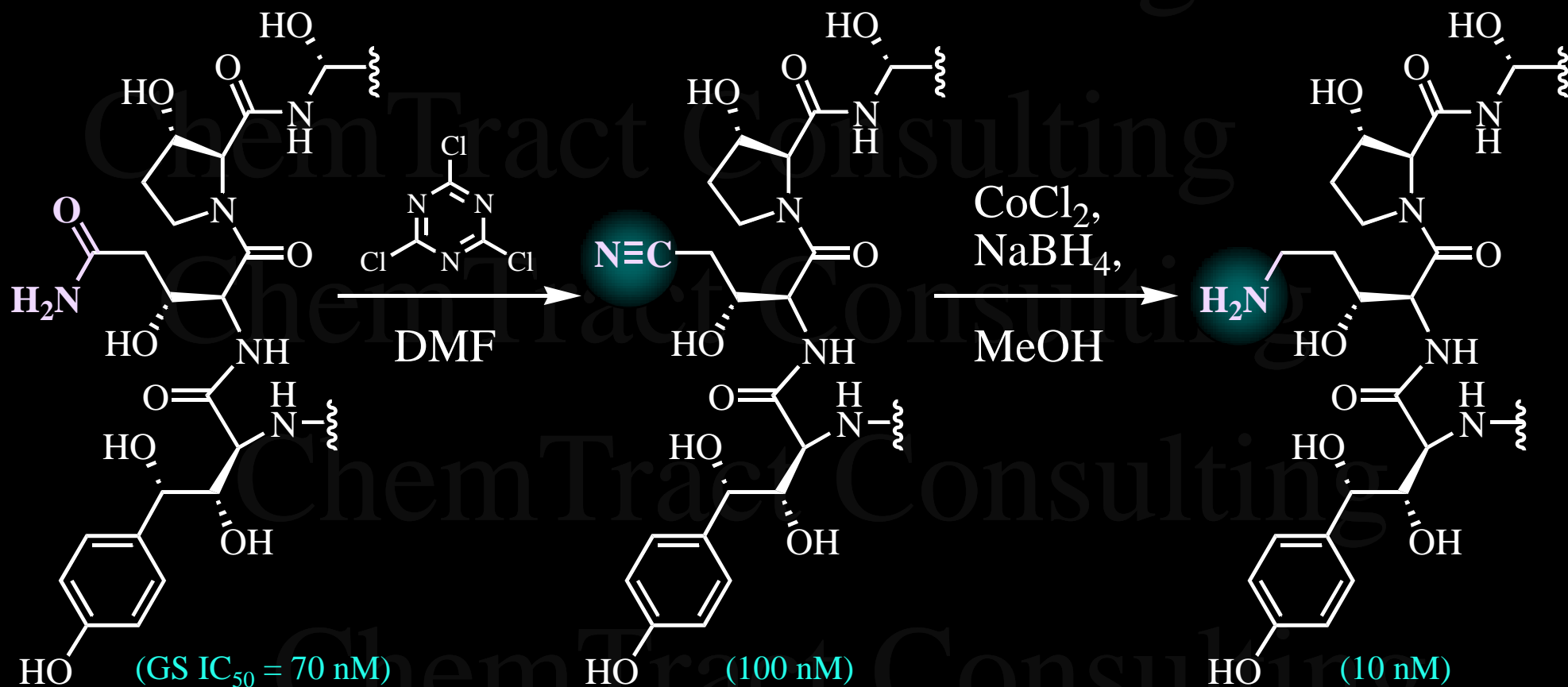
	-OH	-OCH ₂ CH ₂ NH ₂ '589	-OCH ₂ CH ₂ CH ₂ NH ₂	-OCH ₂ (CH ₂) ₄ CH ₂ NH ₂	-OCH ₂ (CHOH) ₄ CH ₂ NH ₂	
# of C's in tether	--	2	3	6	6	
GS IC ₅₀	70	10	20	50	16	(nM)
MFC						
<i>C. albicans</i>	0.25	0.125	0.25	2	1	(μg/mL)

Hypothesis: Cationic Lipopeptide Is Better Positioned in Plasma Membrane to Interact With GS Enzyme





Cationic Pneumocandins



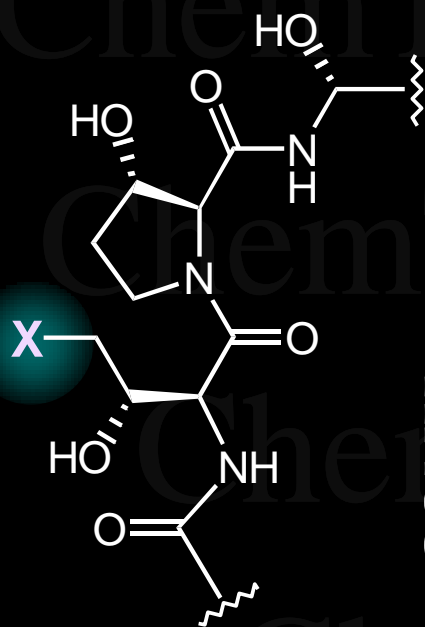
373

- Intrinsic water solubility
- Very potent!

(30% yield over 2 steps)

R. Zambias

3-OH Ornithine SAR



-CONH₂

-CH₂NH₂ '373

-CH₂NMe₃⁺

-CH₂NHGly

-CH₂NHOrn

-CH₂NHAc

GS IC₅₀

70

10

9

15

3

300 (nM)

Net Charge

0

+1

+1

+1

+2

0

MFC

C. albicans

0.25

<0.06

0.5

0.5

<0.06

4 (μg/mL)

TOKA (ED₉₉)

3

0.2

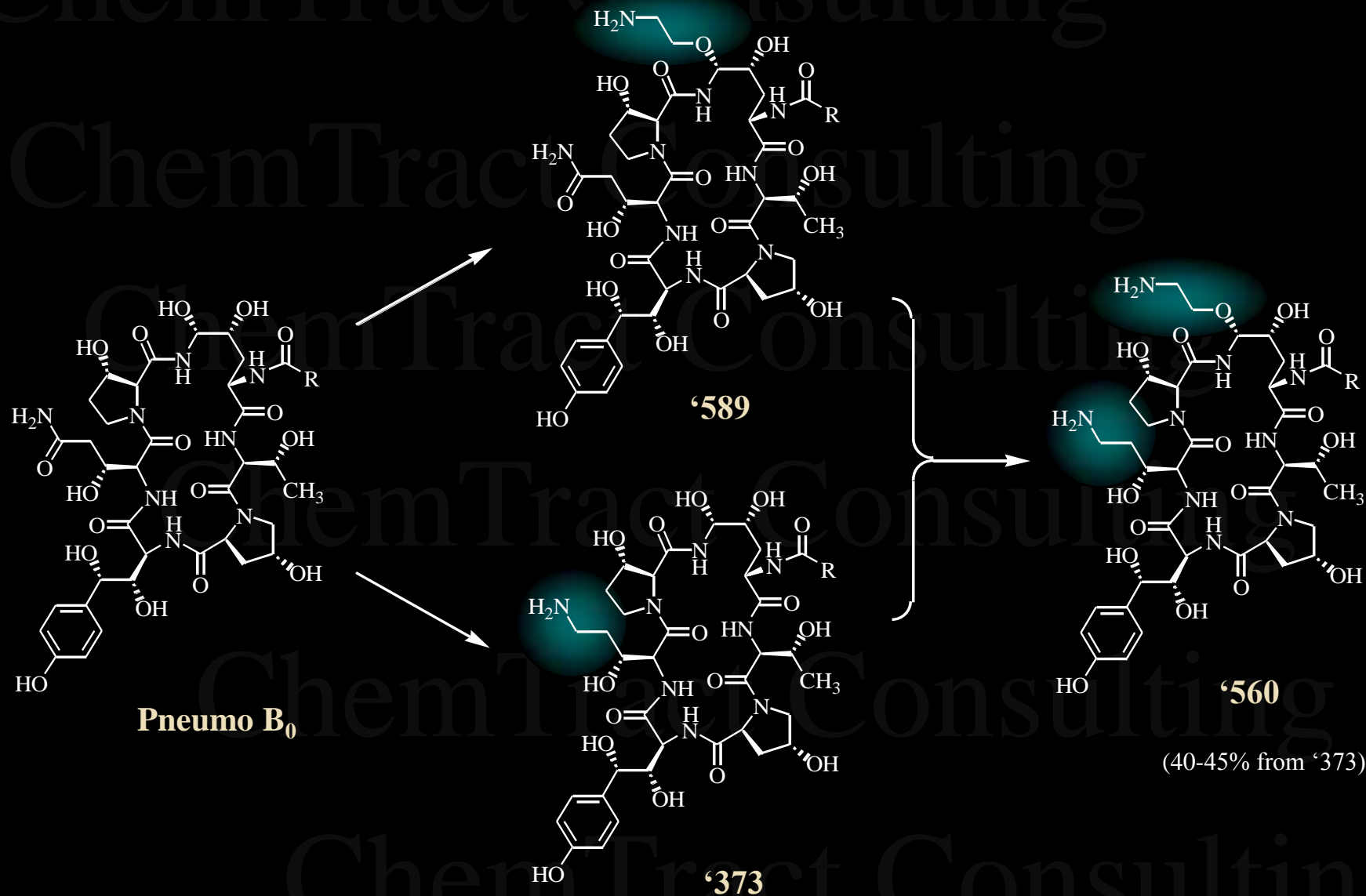
0.2

0.3

0.2

10 (mg/kg)

Combination Analog



Comparative Activities

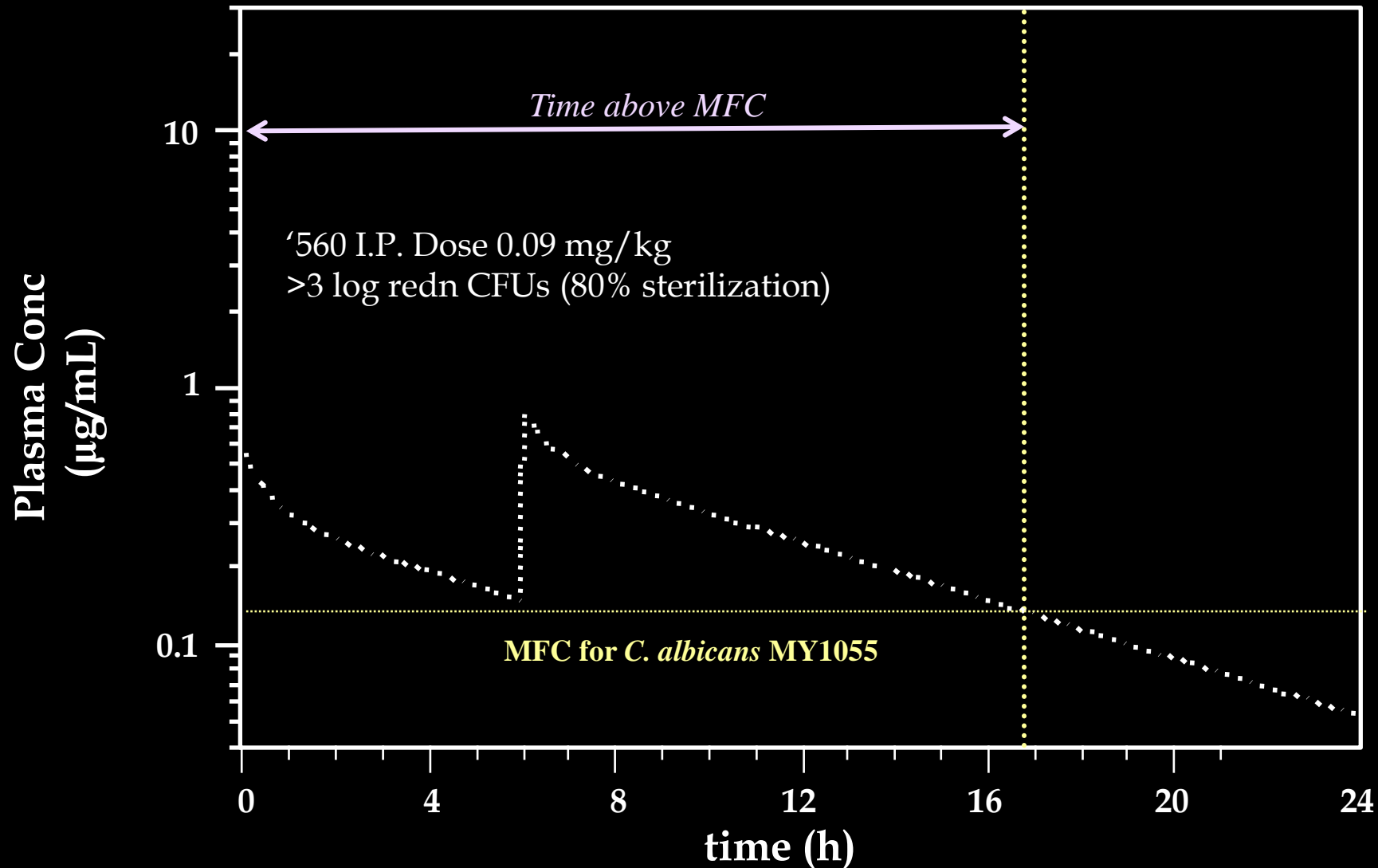
<u>Assay</u>	<u>Pneumo</u>	<u>'560</u>	
GS IC ₅₀	70	1	(nM)
<hr/>			
MFC			
<i>C. albicans</i>	0.25	<0.06	($\mu\text{g/mL}$)
<i>C. parapsilosis</i>	4	0.125	
<i>A. fumigatus</i> *	1	0.015	
<hr/>			
TOKA (ED _{99.9})	6	0.09	(mg/kg)
% MB cure	(20%)	(80%)	
<hr/>			
ASP (ED ₅₀)	>20	0.03	(mg/kg)

*MEC

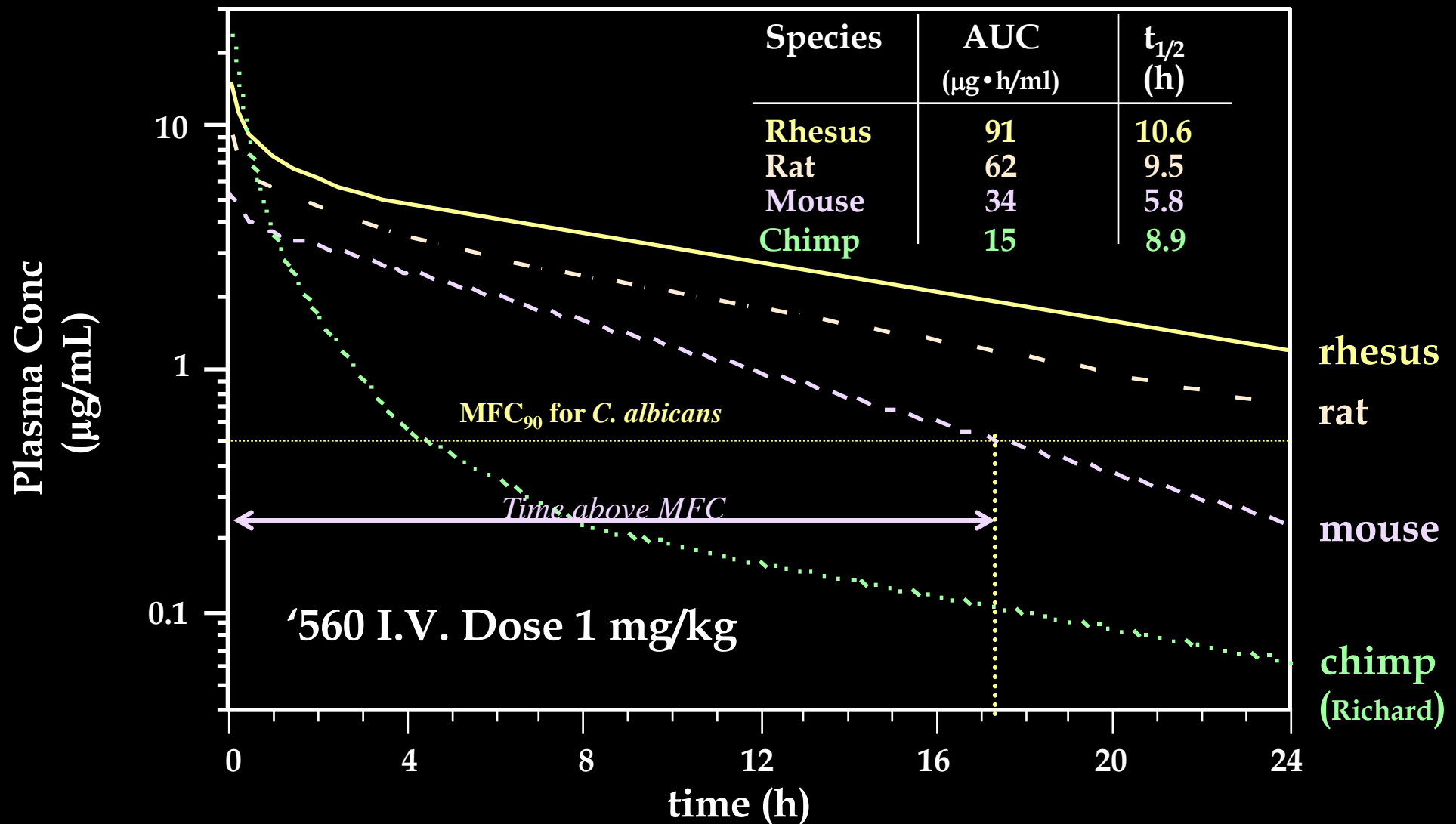
Increasing potency



TOKA Efficacy: Pharmacodynamic Analysis



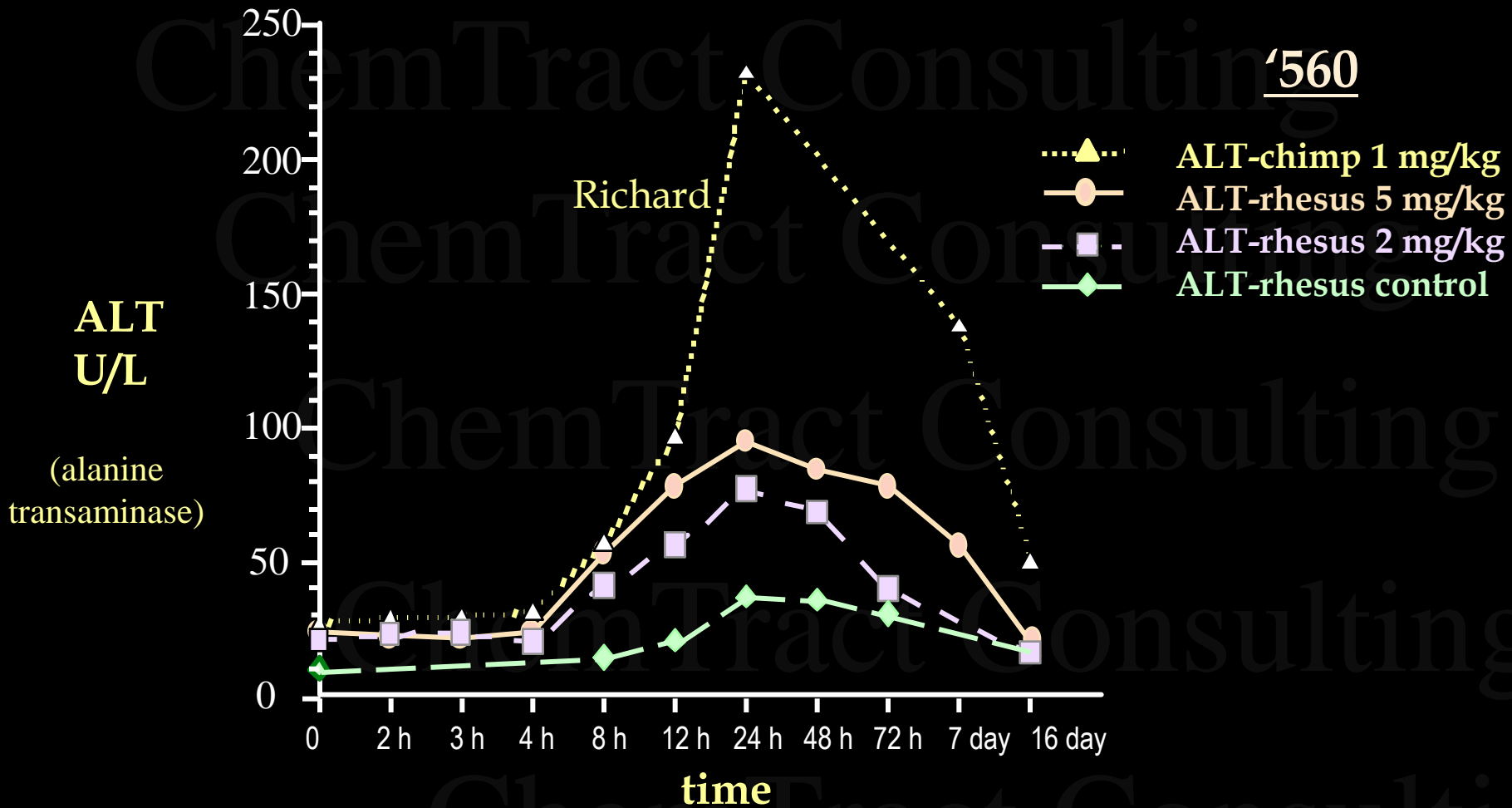
Comparative Pharmacokinetics



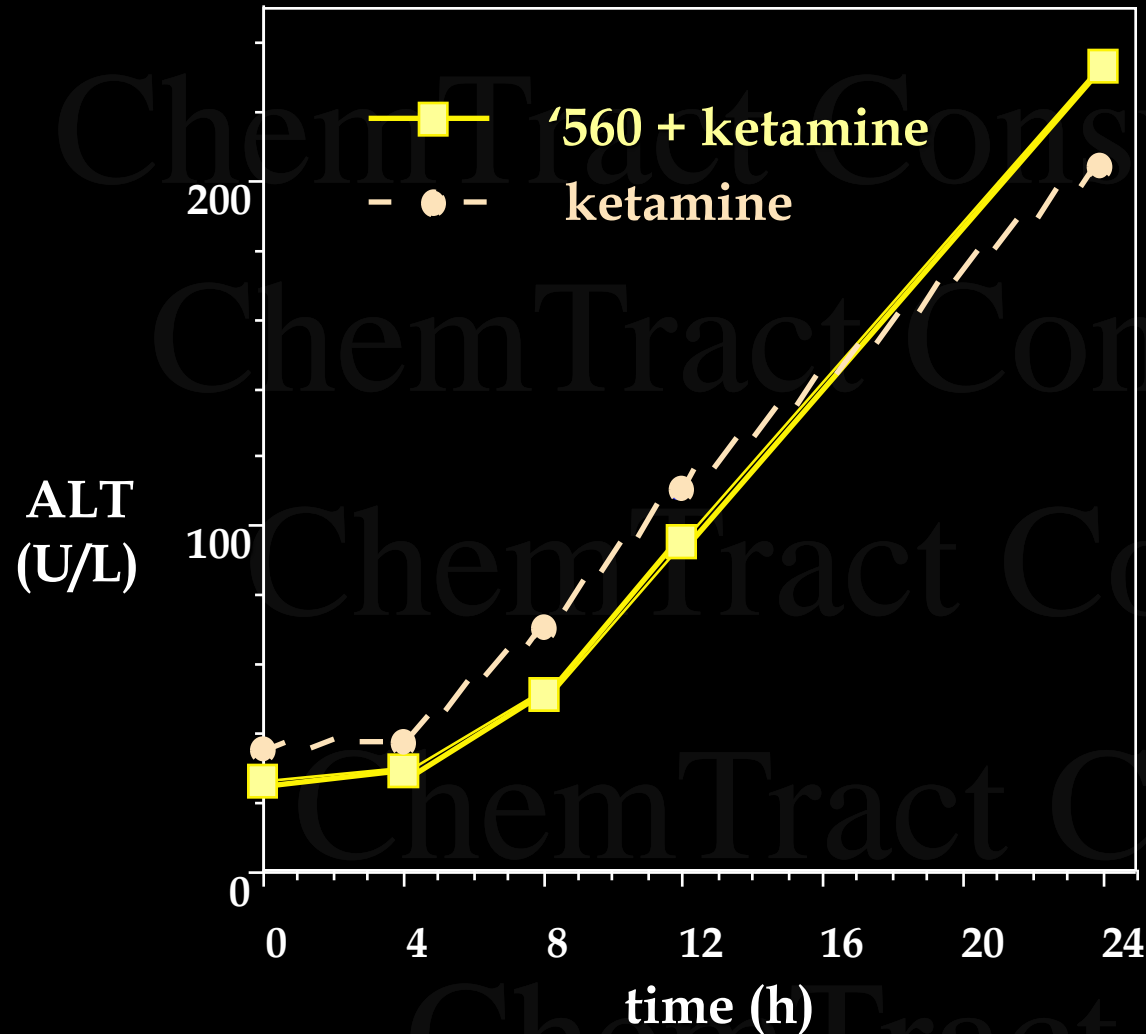
An Additional Issue Arose!!!

- Chimpanzee (Richard) that received '560 was not eating at regular mealtimes and was lethargic
- Blood chemistry was performed on PK samples and followup blood samples out to 16 days...

Apparent Hepatotoxicity!



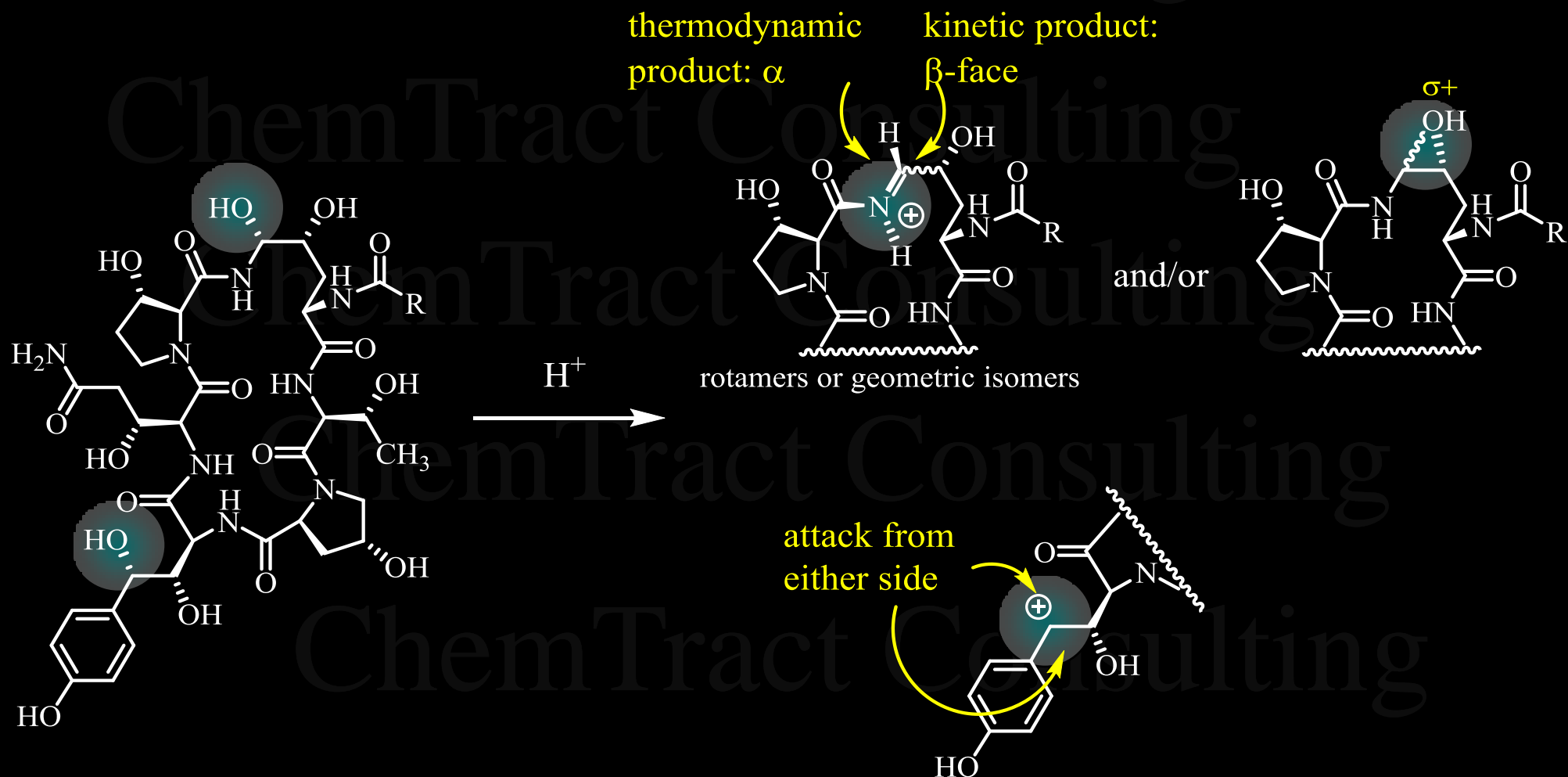
ALT Response in 'Richard'



- Subsequent studies in 'Ron' and 'Timmy' showed no abnormal LFTs

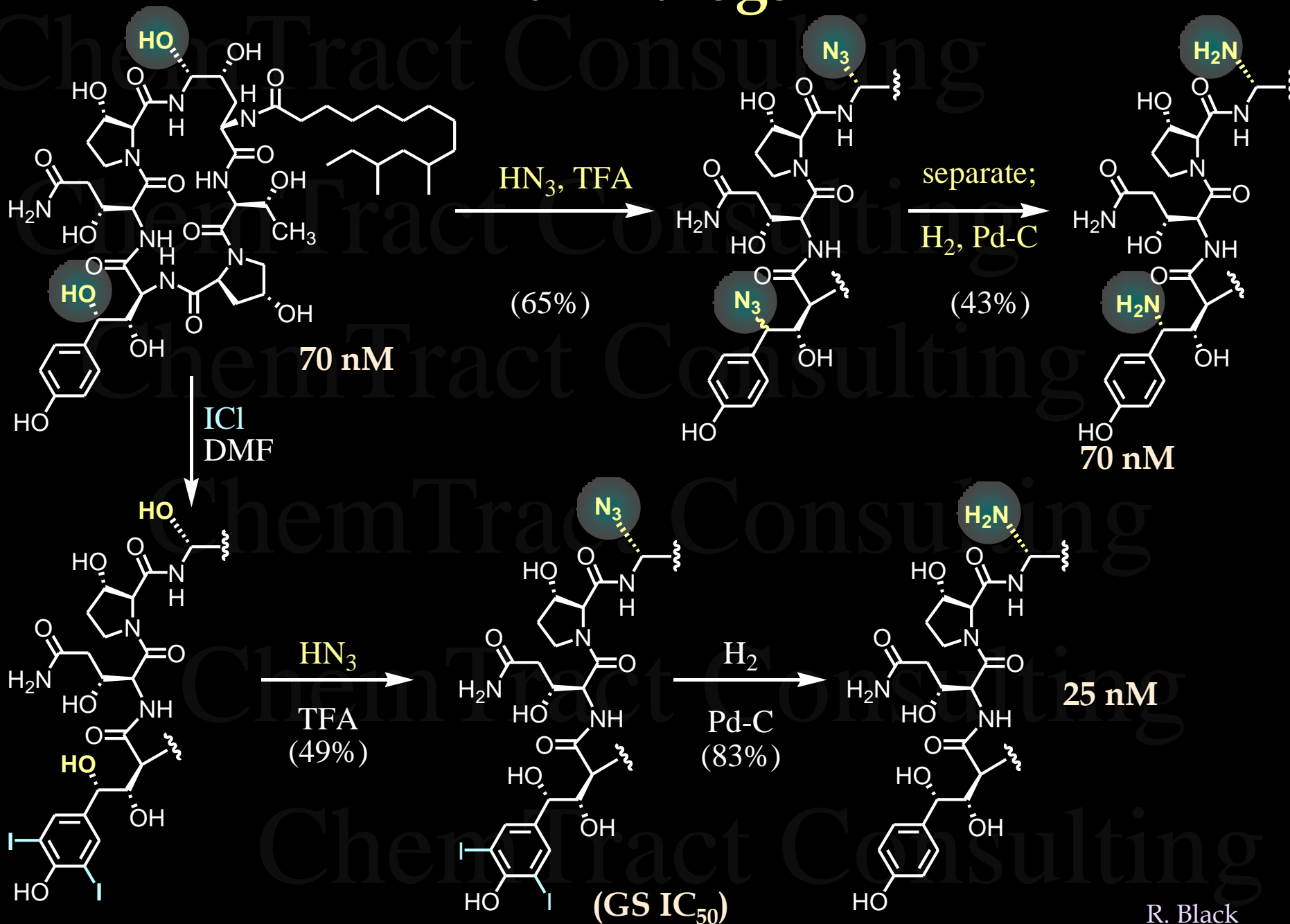
But... '560 was now tainted!

Ionization of Hemiaminal and Benzylic Hydroxyls

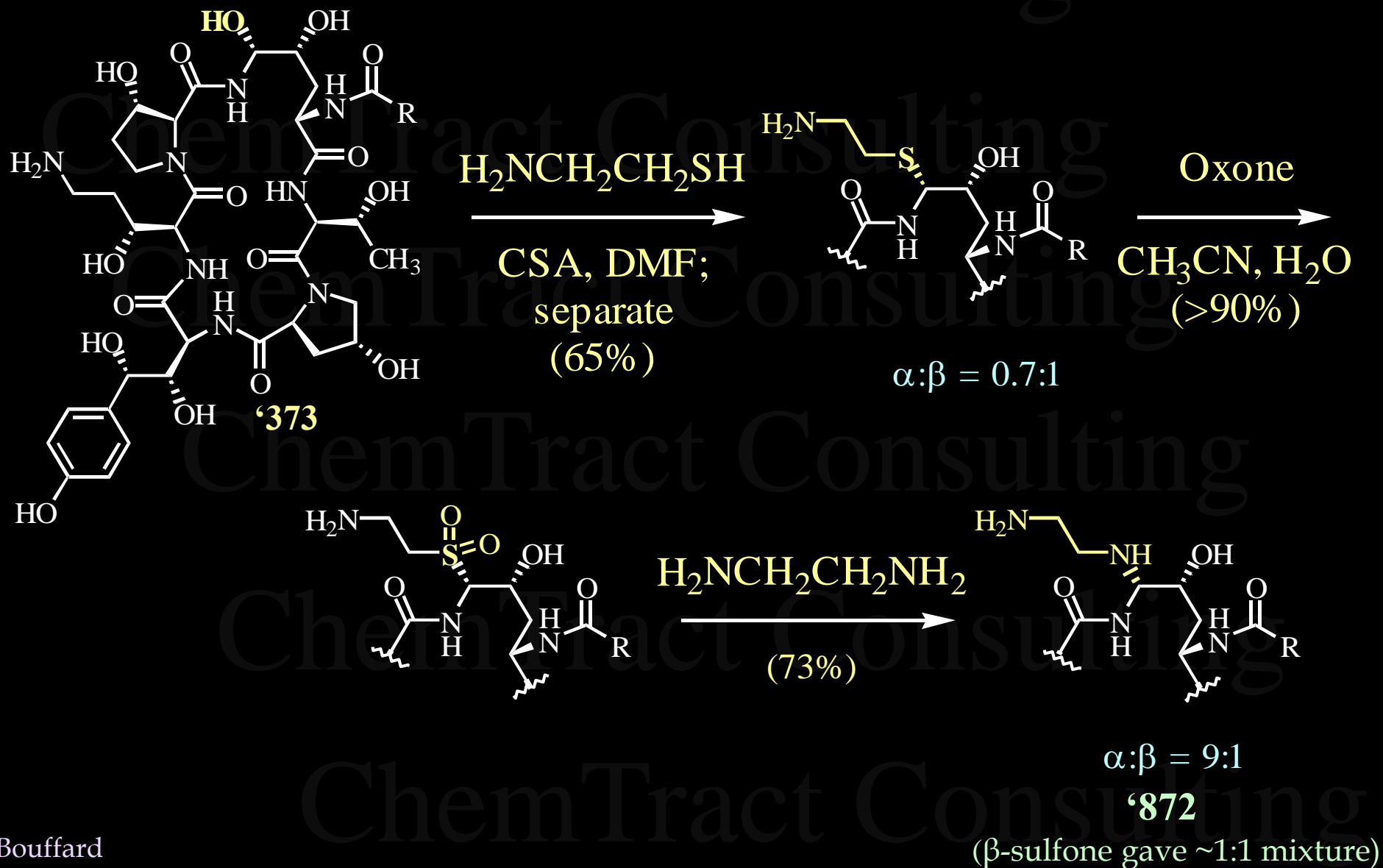


Ionization at benzylic (C4-hTyr) position slower than at hemiaminal (C5-diOHorn)

Aza Analogs



Synthesis of Aminoethylaminal



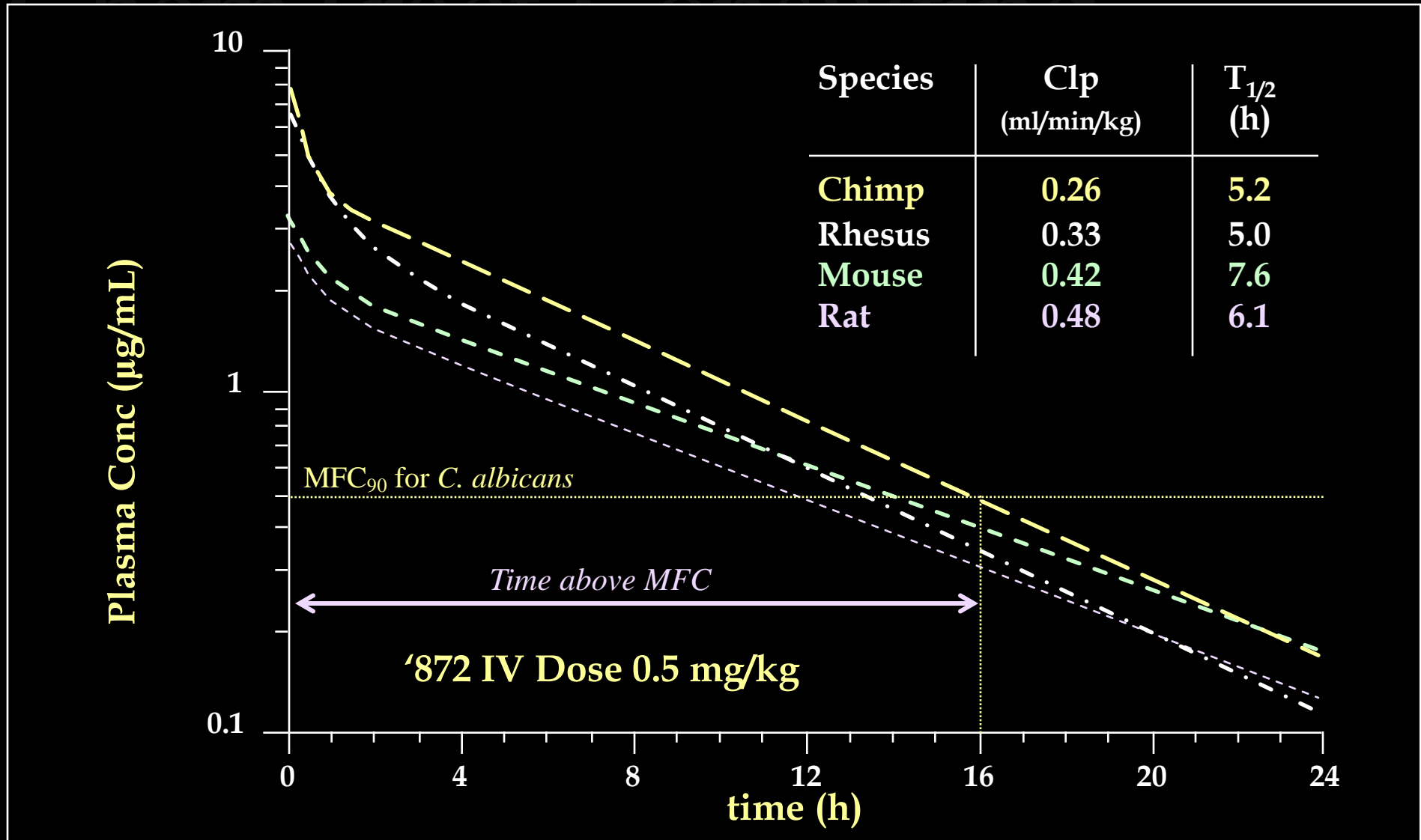
Comparative Activity



<u>Assay</u>	0.6	1	<u>AmB</u>	
GS IC₅₀			NA	(nM)
MFC				
<i>C. albicans</i>	0.125	0.125	0.25	(μg/mL)
<i>C. parapsilosis</i>	0.25	0.125	0.25	
<i>A. fumigatus</i>	0.008*	0.015*	1	
TOKA ED₉₉	0.027	0.045	0.013	(mg/kg)
ASP ED₅₀	0.05	0.03	0.06	(mg/kg)
Acute Tox	50	30	4	(mg/kg)

*MEC

Pharmacokinetics in Four Species



- Supports once daily dosing

ChemTract Consulting

ChemTract Consulting

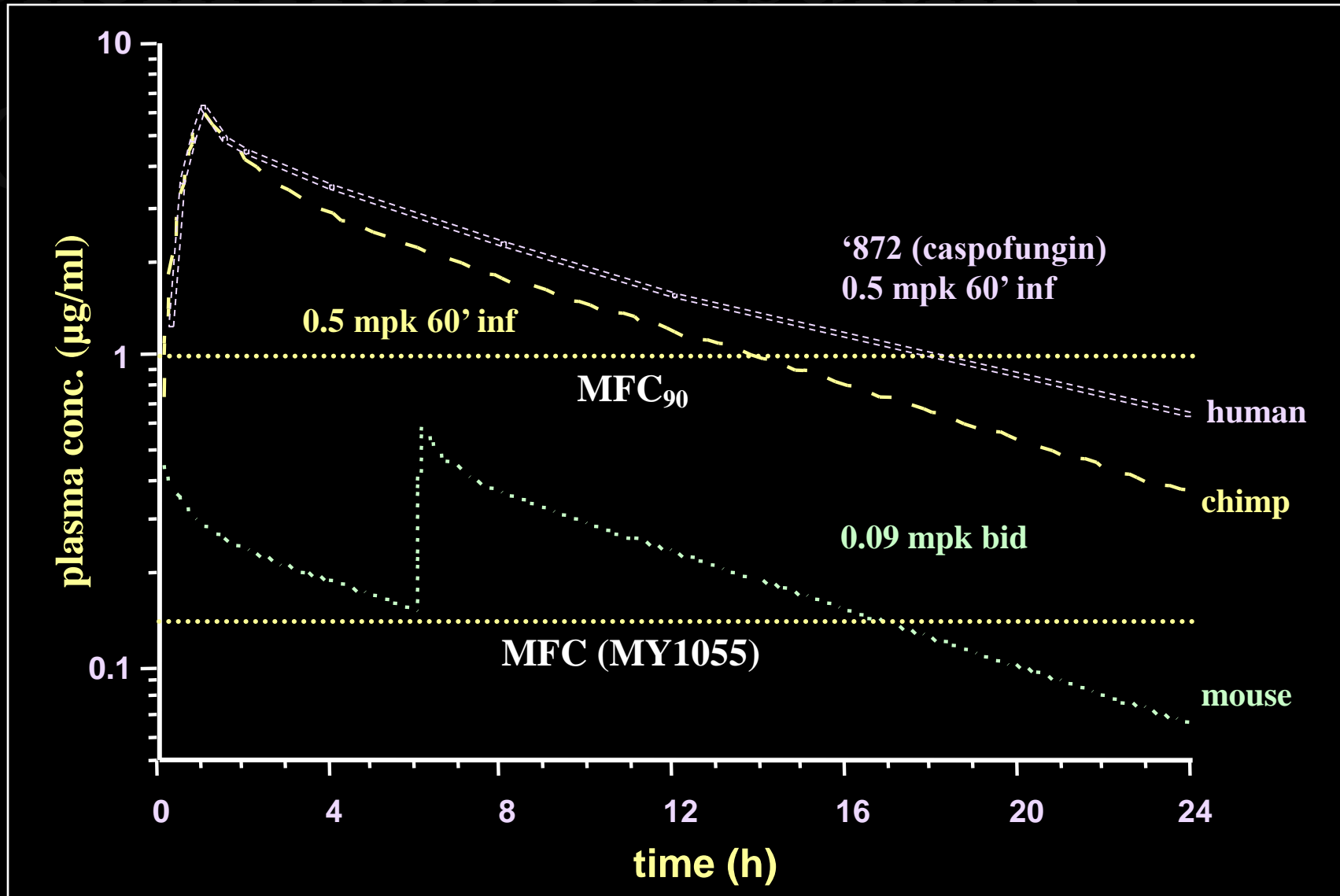
Select Clinical Results

ChemTract Consulting

ChemTract Consulting

ChemTract Consulting

Phase I Pharmacokinetics



Clinical Development Program

Phase II Dose Ranging Studies
Esophageal/Oropharyngeal Candidiasis
35, 50, 70 mg/d Caspofungin vs AmB

Dose Selection

50 mg/d

70 mg X 1, then 50 mg/d

Candida Esophagitis

Caspofungin vs.
Fluconazole

Invasive Candidiasis

Caspofungin vs.
Amphotericin B

Salvage Aspergillosis

Caspofungin

Esophageal Candidiasis Study Design

- Double-blind, multi-center, dose ranging study
 - caspofungin 50mg or 70mg/d, vs.
 - amphotericin B 0.5mg/kg/d
- 128 patients with documented *Candida* esophagitis by endoscopy for whom IV therapy was appropriate
- Treatment duration 14 days
- Response assessed 14 days post-therapy
 - resolution of symptoms and
 - favorable endoscopic response: 2-grade reduction or reduction to Grade 0
- Safety assessed during treatment, and for 14 days after the end of therapy

Esophageal Candidiasis Disease Grading

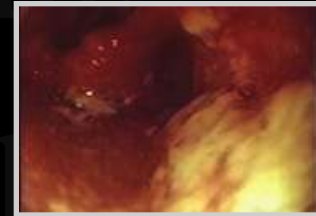
0 Normal esophagus Grade 0



1/2 Rare scattered individual plaques, each <2mm Grade 1



1 Scattered individual plaques, each >2mm Grade 1



2 Plaques >2mm in size covering >50% of the esophageal mucosa Grade 2



3 Confluent plaques circumferentially coating at least 50% of the mucosa Grade 3



4 Circumferential plaques with narrowing despite insufflation Grade 4

Efficacy Results

	Caspofungin 50mg N=46	Caspofungin 70mg N=28	AmB 0.5mg/kg N=54
Endoscopic response	74%	89%	63%
Microbiologic eradication	76%	89%	61%

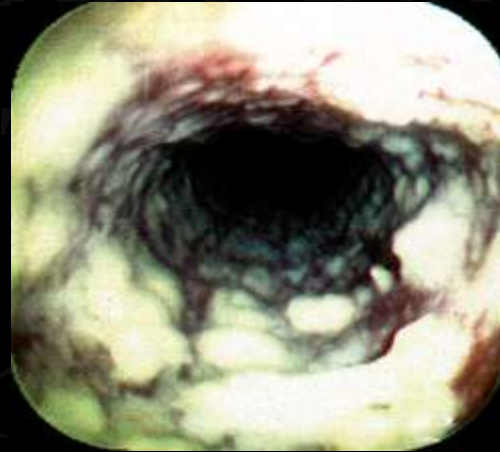
CANCIDAS®

Treatment of Esophageal Candidiasis

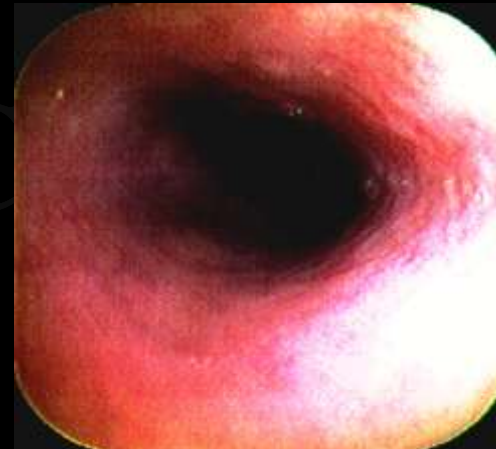
Before

After

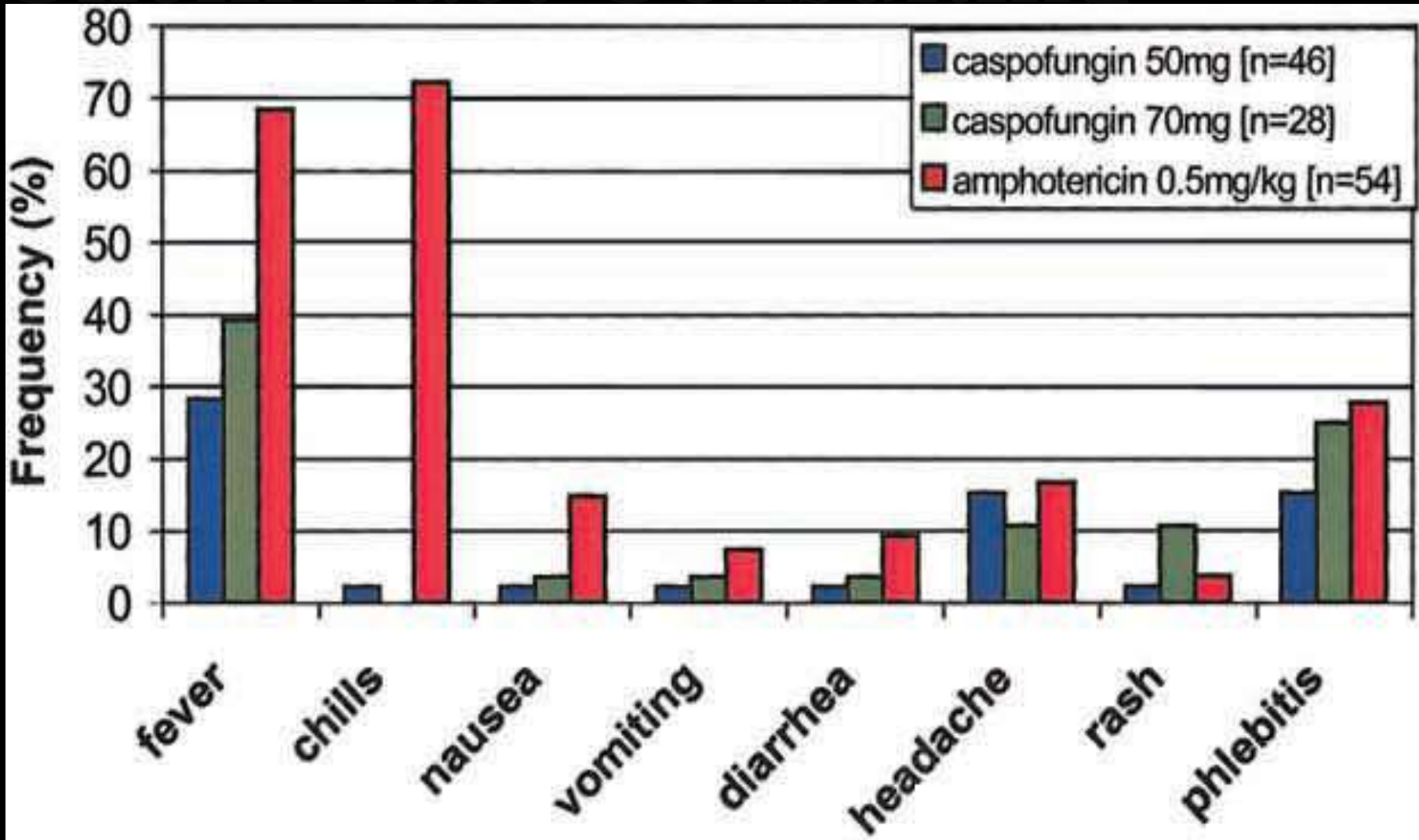
Patient 1



Patient 2



Safety



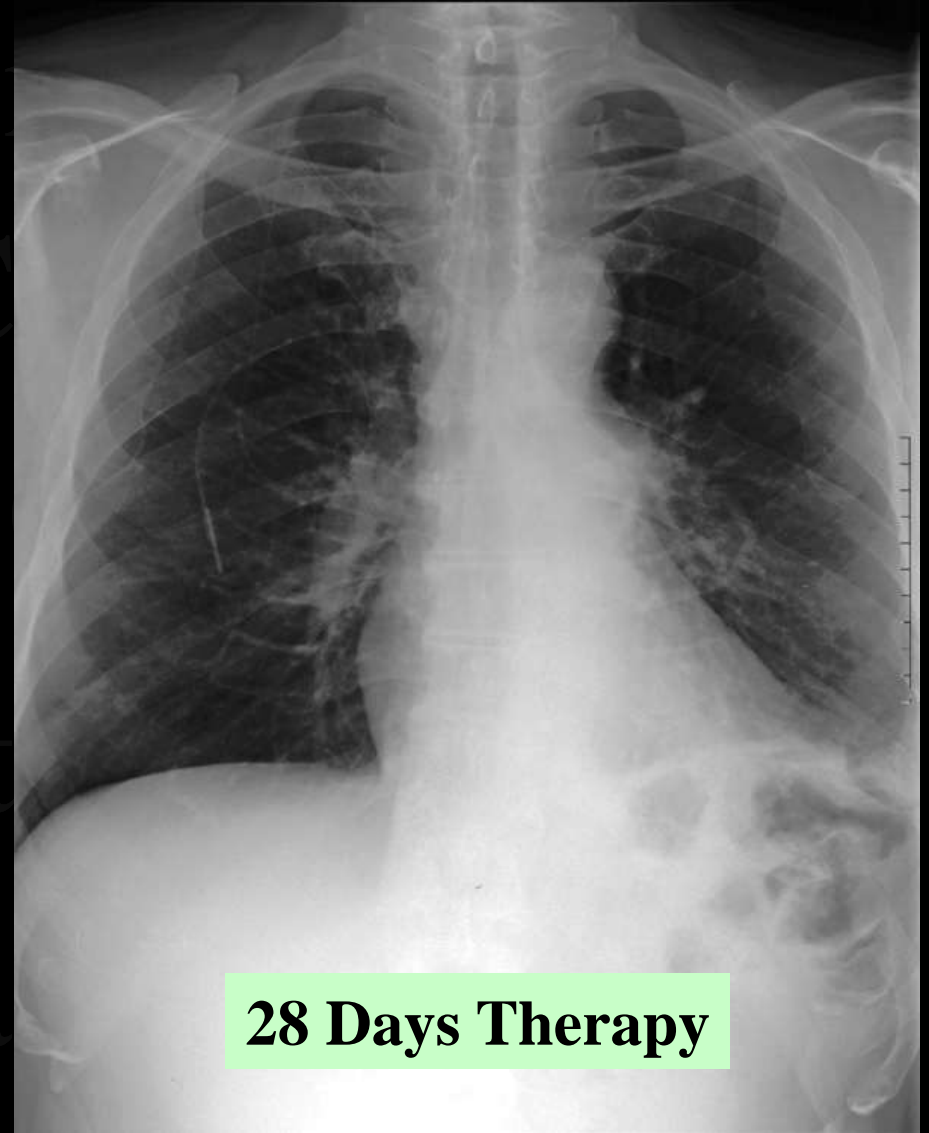
Caspofungin Salvage Aspergillosis Study Design

- Multi-center, open-label, non-comparative study
 - Caspofungin 70 mg qd X 1, followed by 50 mg qd
- Diagnostic criteria
 - Documented invasive aspergillosis, AND
 - Meet criteria as refractory to or intolerant of standard therapy
- Definition of response
 - Favorable response: Complete or Partial Response
 - Unfavorable response: Failure, Stable disease
- First 90 cases reviewed by independent Expert Panel
 - Favorable responses in 37/83 (45%)

CANCIDAS® (caspofungin acetate) Treatment of Pulmonary Aspergillosis



Pre-Treatment



28 Days Therapy



FDA Approval on January 26, 2001

Caspofungin Timeline

- **1985:** Pneumocandins first identified and isolated
- **1989:** Medicinal chemistry program initiated
- **1992:** L-872 first synthesized in lab
- **1993:** Approved for development within Merck
- **2001:** First echinocandin approved in the US and EU
 - Indicated for treatment of invasive aspergillosis in patients refractory to or intolerant of standard antifungal therapy
- **2002:** Indication for treatment of esophageal candidiasis
- **2003:** Indication for treatment of candidemia and other *Candida* infections: peritonitis, intra-abdominal abscess, & pleural space infections
- **2004:** Indication for treatment of empirical therapy for presumed fungal infections in febrile, neutropenic patients
- **2006:** *Cancidas became the #1 IV antifungal worldwide: has saved the lives of thousands of patients*

Merck Acknowledgments

- Natural Products Chemistry
- Parasite Biochemistry and Cell Biology
- Medicinal Chemistry
- Fermentation Microbiology
- Antibiotic Discovery and Development
- Infectious Disease Research
- Drug Metabolism
- Pharmacology
- Comparative Medicine
- Process Chemistry
- Bioprocess R&D
- Toxicology
- Clinical Research
- Regulatory Affairs
- Marketing



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