K20, Novel Antifungal Aminoglycoside

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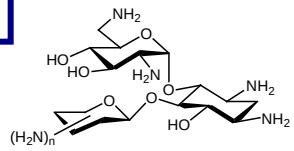
Research Overview

Funding: NIH, DOT, USTAR and companies

Antibacterial
Antifungal
SMA therapeutics

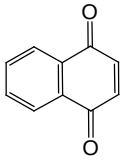
UtahState University

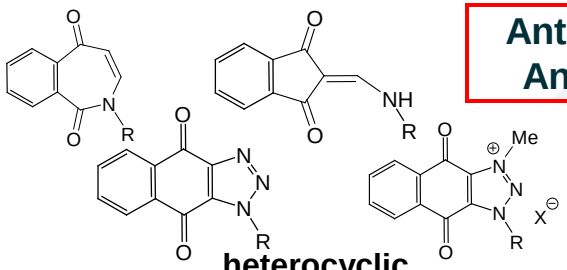
Carbohydrates



aminoglycosides

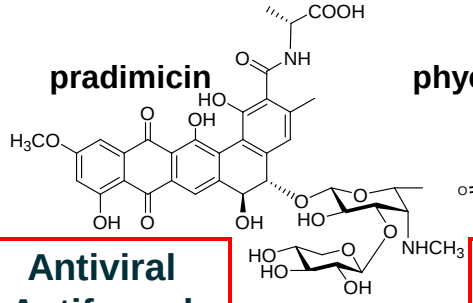
Small molecule



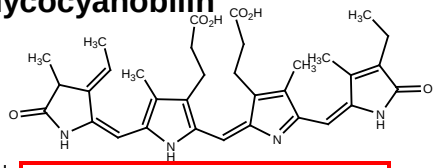


heterocyclic

Natural products

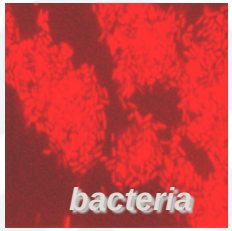


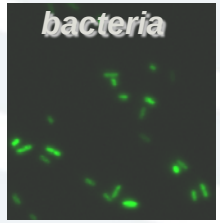
pradimicin



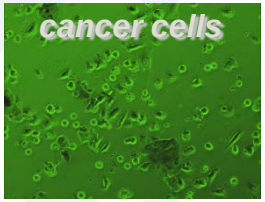
phycocyanobilin

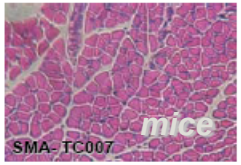
Anticancer





Antibacterial
Anticancer

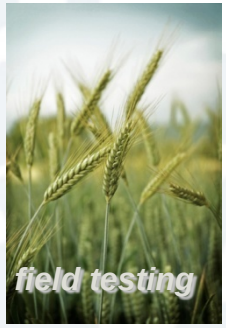




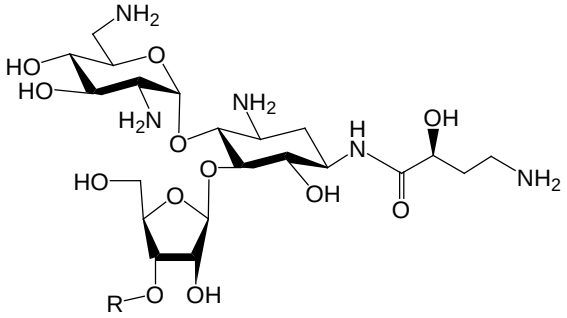
Antiviral
Antifungal

Anti-inflammatory

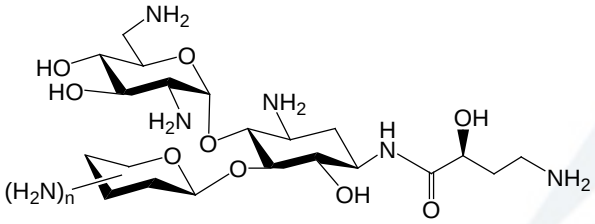
Biofuel



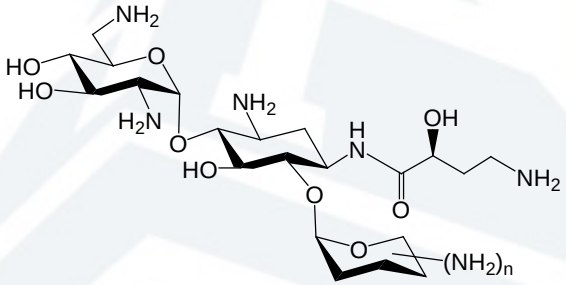
Library of Synthesized Aminoglycosides



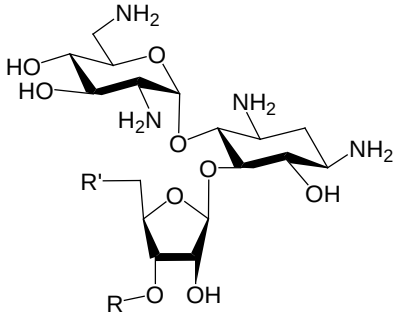
neomycin analogs with N-1 AHB (2)



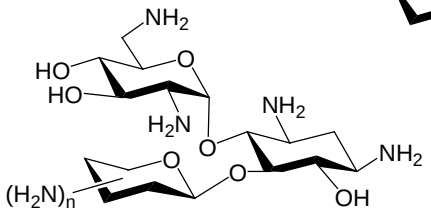
pyranmycin with N-1 AHB (5)



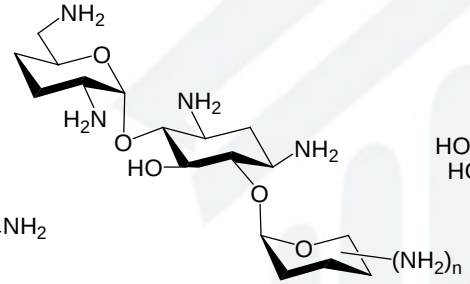
kanamycin analogs with N-1 AHB (6)



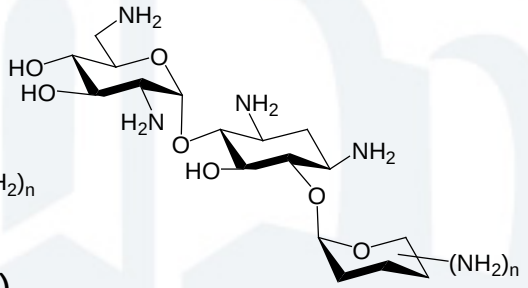
neomycin analogs with various R' (>30)



Pyranmycin (>40)



kanamycin analogs with 3',4'-dideoxygenation (8)



kanamycin analogs (>40)

( ): number of aminoglycosides synthesized

History: Six Years Ago

Journal of Applied Microbiology 2005, **99**, 836–843 doi:10.1111/j.1365-2672.2005.02684.x

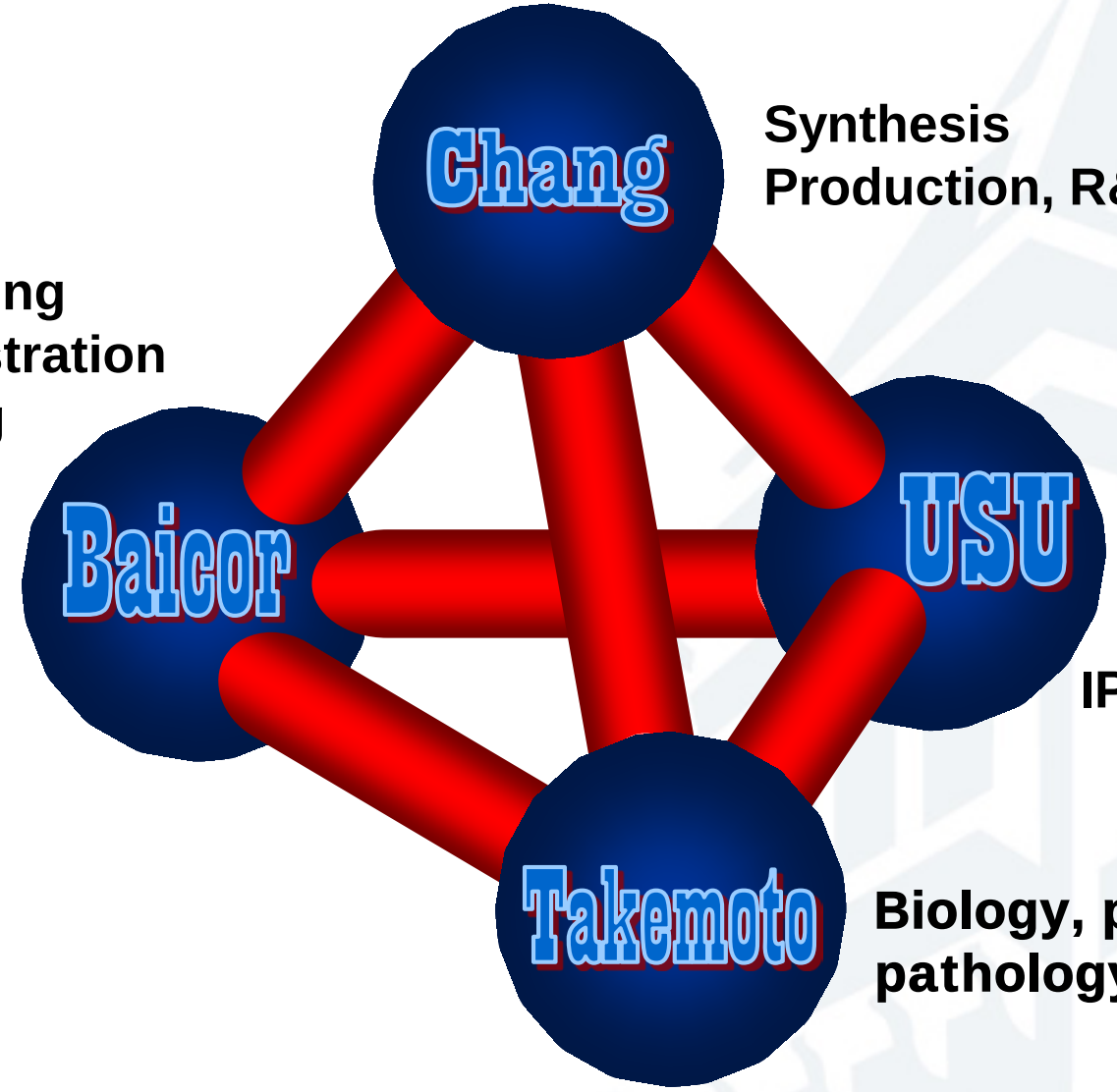
Activity of some aminoglycoside antibiotics against true fungi, *Phytophthora* and *Pythium* species

H.B. Lee[1]*, Y. Kim[2]*, J.C. Kim[3], G.J. Choi[3], S.-H. Park[4], C.-J. Kim[4] and H.S. Jung[1]

[1]Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul, [2]Division of Biotechnology, The Catholic University of Korea, Puchon, [3]Biological Function Research Team, Korea Research Institute of Chemical Technology, Daejeon, and [4]Laboratory of Antioxidants, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

"Certain classical aminoglycosides like streptomycin, neomycin B, paromomycin inhibit important crop pathogenic fungal oomycetes…"

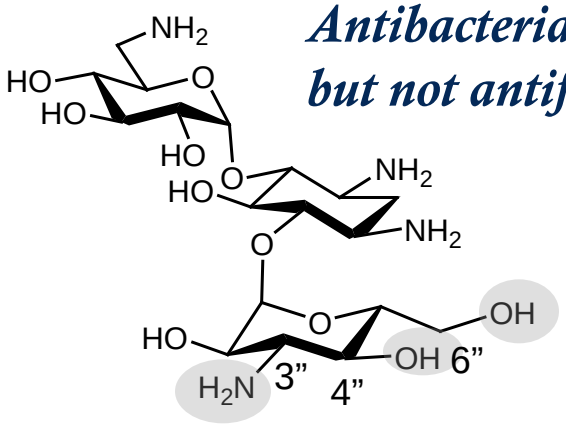
Research Model



Chemical Modification Can Change the Mode of Action

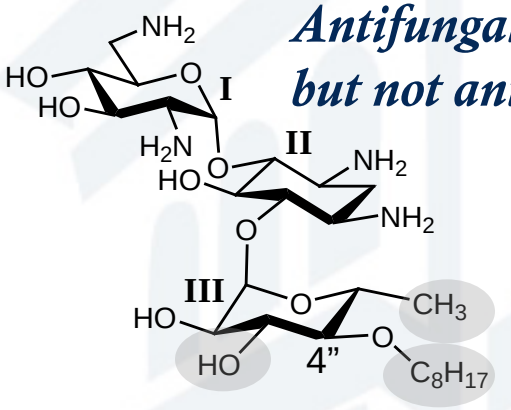
New method for reviving old drug

Turning aminoglycoside from antibacterial to antifungal



Antibacterial but not antifungal

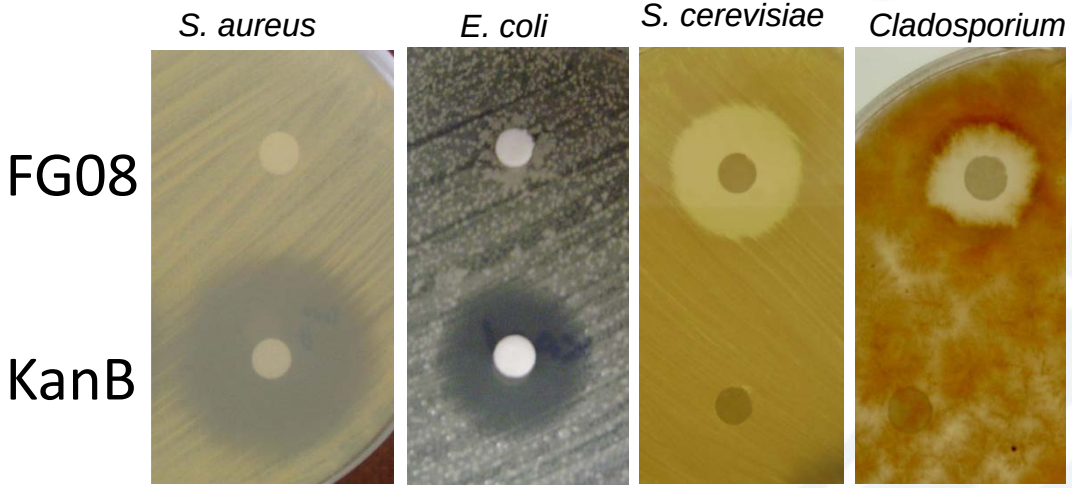
Kanamycin A



Antifungal but not antibacterial

FG08

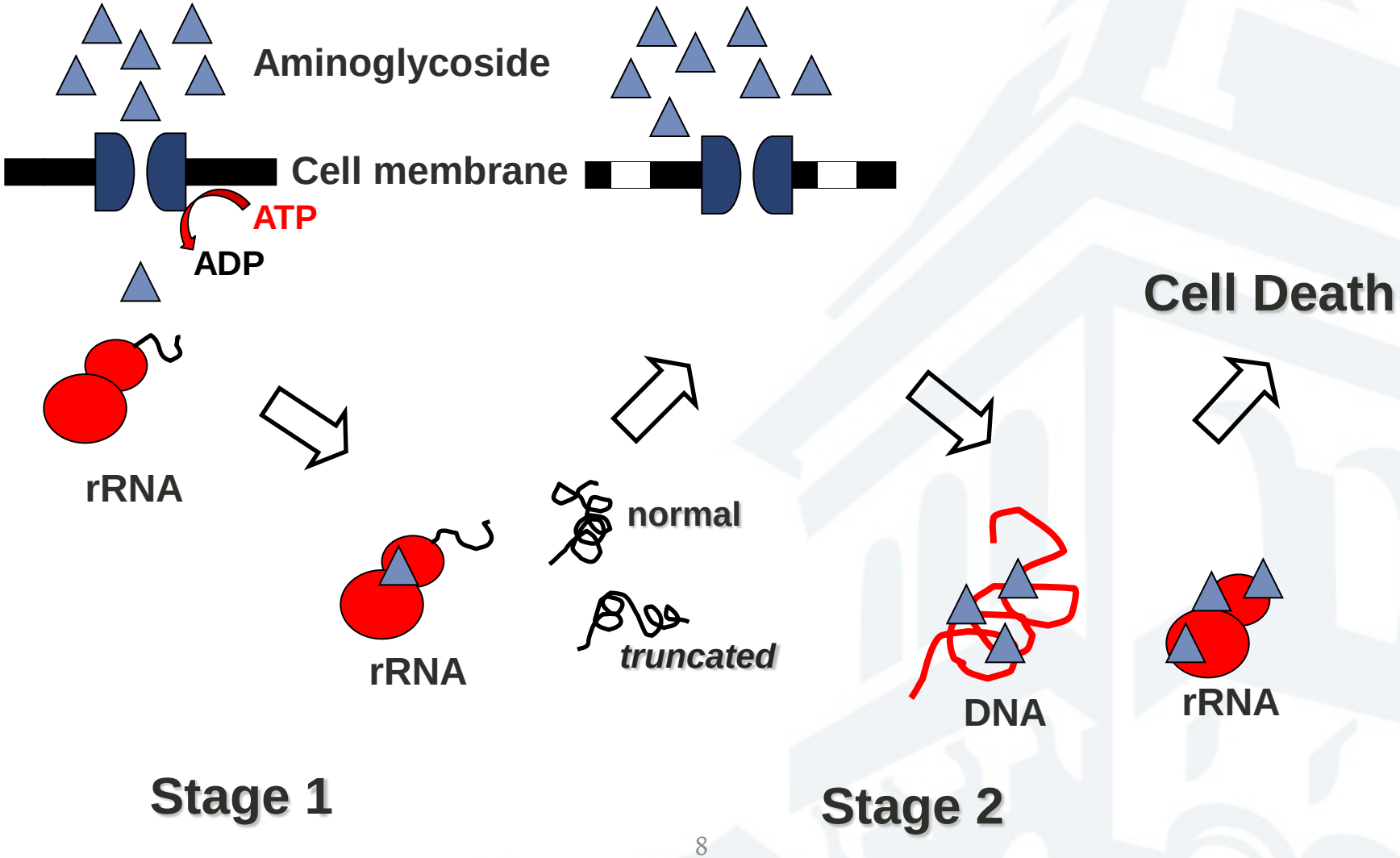
FG08, Novel Antifungal Agent



Fungi	Diseases caused	MIC (µg/mL)
Fusarium oxysporium	Wilt disease	7.8
Ulocladium sp.	Human allergen	7.8
Pythium irregularis	Damping off	15.12
Rhizopus stolonifer	Bread mold	31.2
Cladosporium sp	Tomato leaf mold	31.25
Botrytis sp.	Soft-rot of vegetables and fruits	31.25
Fusarium graminearum	Fusarium head blight (barley, wheat, etc)	15.6
Candida albicans	Human pathogen	15.6
Phoma sp.	Human allergen	31.25

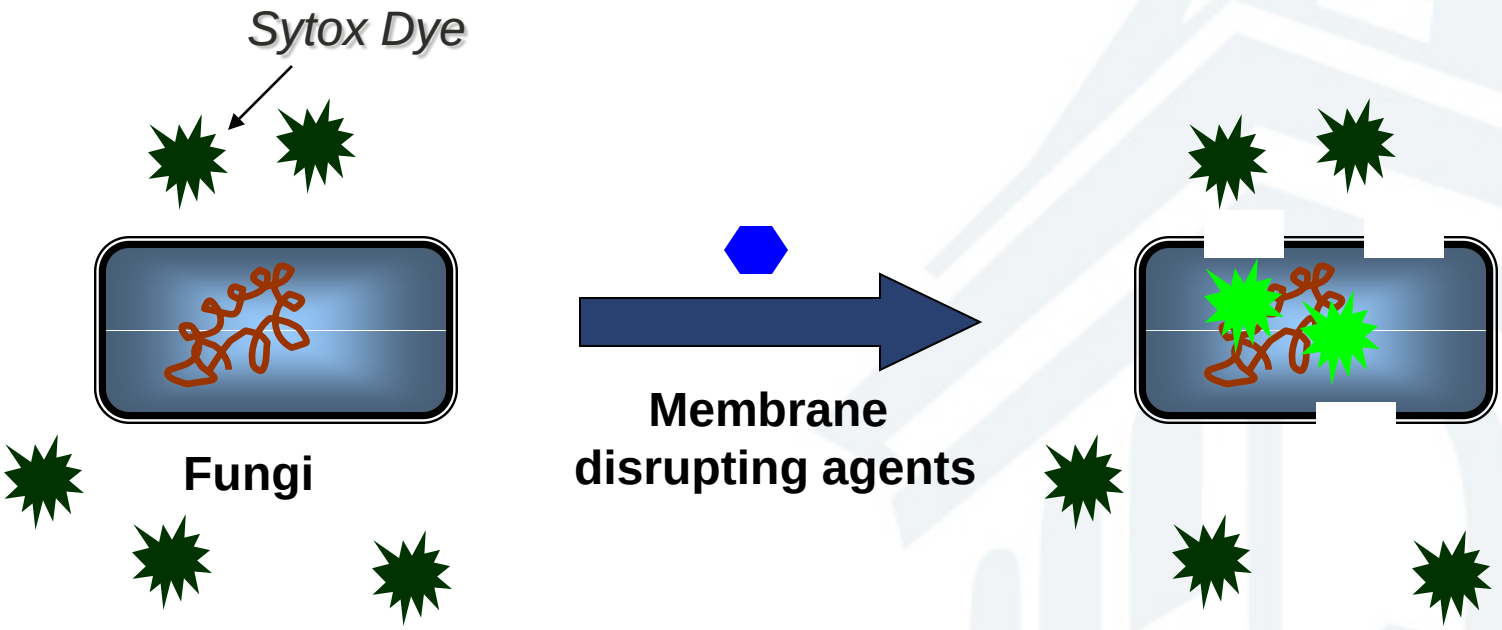
Chang, C.-W. T.; Fosso, M.; Kawasaki, Y.; Shrestha, S.; Bensaci, M. F.; Wang, J.; Evans, C. K.; Takemoto, J. Y. *J. Antibiot.* **2010**, *63*, 667-672.

Aminoglycosides: Traditional Mode of Action



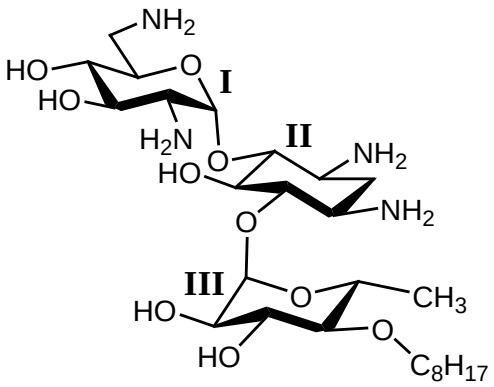
How Does FG08 Kill Fungi?

Application of Sytox Dye



Green fluorescence indicates membrane disruption.

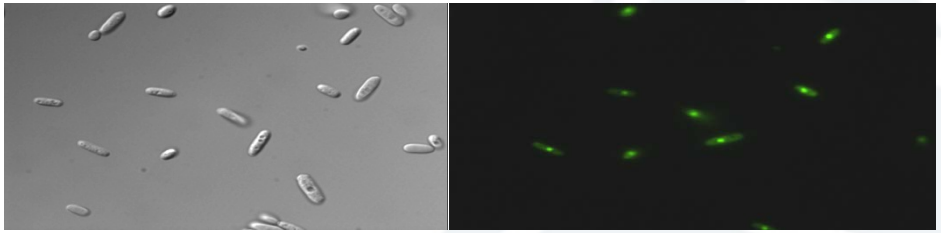
Effect of Octyl (C8) group



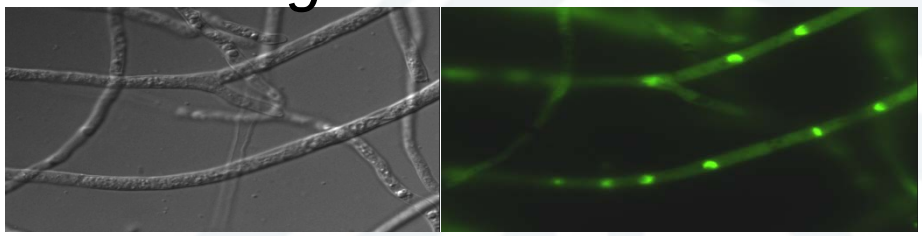
FG08

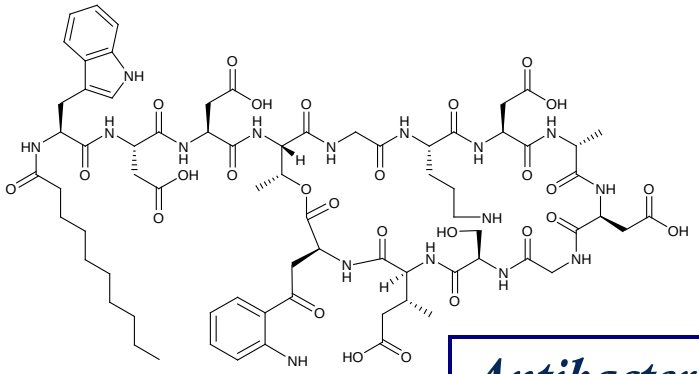
Membrane-disrupting

C. albicans



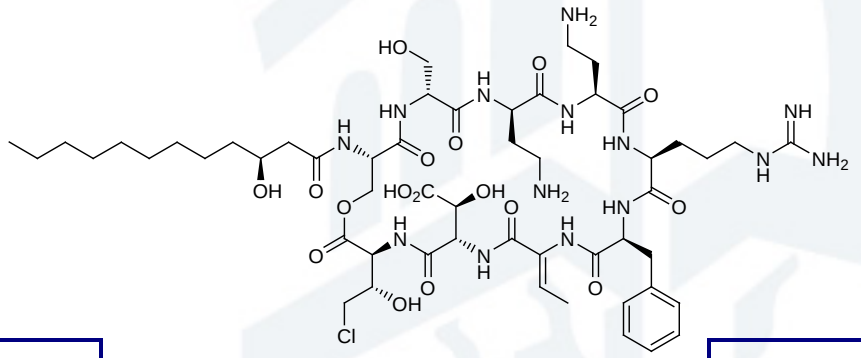
F. graminearum





daptomycin

Antibacterial

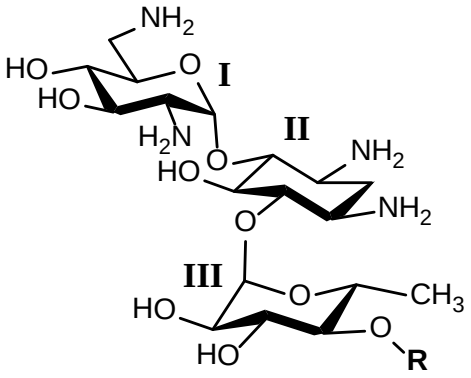


syringomycin

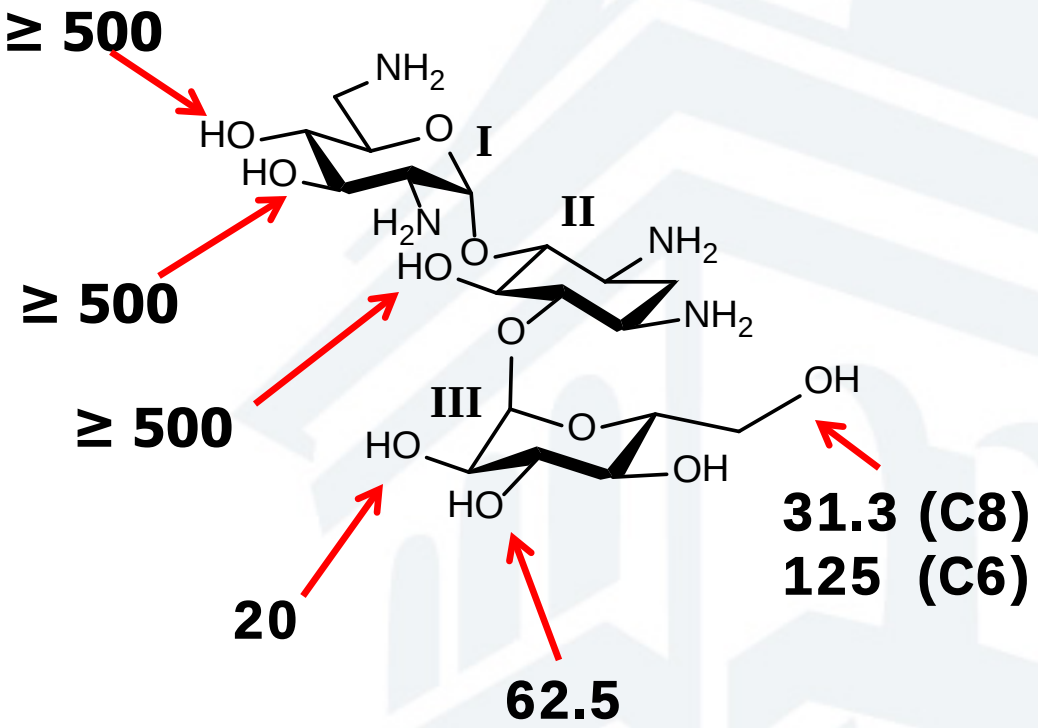
Antifungal

Alkyl Mapping (C8) with FG08 Analog

MIC (μg/mL) against *Fusarium graminearum*



R =	MIC (μg/mL)
C_8H_{17} (FG08)	7.8
C_4H_9 (FG01)	≥ 250
$C_{12}H_{25}$ (FG02)	≥ 250



Impact of Fungal Pathogens

The annual cost of controlling black Sigatoka of banana caused by *Mycosphaerella fijiensis* in Costa Rica: $49 million

Barrientos, E. *et. al*. Corporación Bananera Nacional, San José, Costa Rica, **1995**.

Economic losses from soybean rust caused by *Phakopsora pachyrhizi* in USA: $240 million to $2 billion

Stokstad, E. *Science* **2004**, *306*, 1672-1673.

The annual economic losses from potato late blight caused by the fungus *Phytophthora infestans* worldwide: $3 billion

McGraw, L. Article from Agriculture Research Service, USDA.

Fungal pathogens destroy >125 million tons/yr of the top five food crops (rice, wheat, maize, potatoes and soybeans) that can feed more than 600 million people.

Fisher, M. C. *et. al. Nature*, **2012**, *484*, 186-194.

Development of new fungicide: close to priceless

“From A Teaspoon To A Ton”

“People don’t really know what it takes to do a synthesis scale-up and produce material on large scale,..”

Ahmed Abdel-Magid, a process chemist at Therachem Research Medilab.

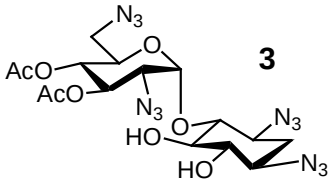
“Without the ability to make a compound on a large scale, it’s essentially *no more than a laboratory curiosity* and it’s not going to be of any great benefit to the public.”

Jaan Pesti, a principal scientist in process chemistry at Bristol-Myers Squibb

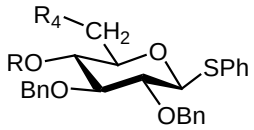
C & EN News September 6, **2010**, *88*, 17-28.

Can FG08 Be Synthesized in Large Quantity?

(a) NIS, TfOH, Et_2O:CH_2Cl_2 (3:1),

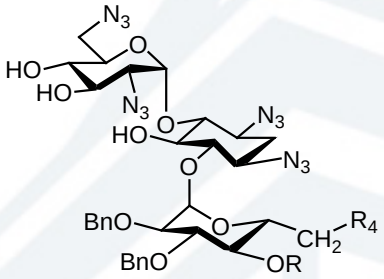


3



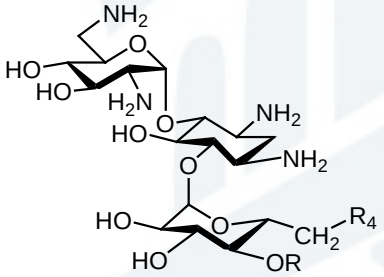
2a R = C_4H_9, R_4 = H
2b R = C_8H_{17}, R_4 = H
2c R = $C_{12}H_{25}$, R_4 = H
2d R = C_8H_{17}, R_4 = OBn

(b) NaOMe, MeOH:THF (5:1).



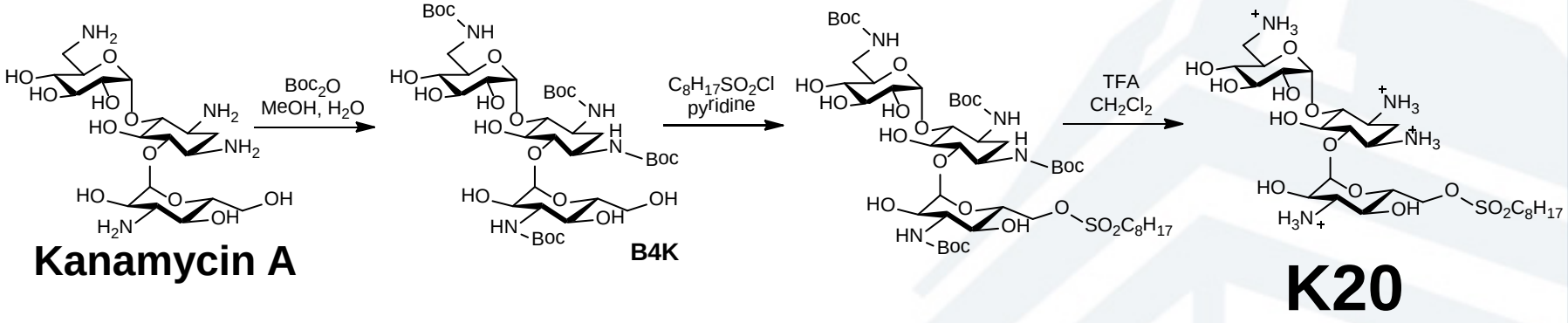
4a R = C_4H_9, R_4 = H
4b R = C_8H_{17}, R_4 = H
4c R = $C_{12}H_{25}$, R_4 = H
4d R = C_8H_{17}, R_4 = OBn

(a) PMe_3, THF, NaOH,
(b) H_2, $Pd(OH)_2$/C, HOAc/H_2O (1/3),
(c) Dowex 1X8-200 (Cl^- form).



FG01 R = C_4H_9, R_4 = H
FG08 R = C_8H_{17}, R_4 = H
FG02 R = $C_{12}H_{25}$, R_4 = H
FG03 R = C_8H_{17}, R_4 = OH

Synthesis of FG08 analog (2nd Generation)



Synthesized in 3 steps (>80% yield)

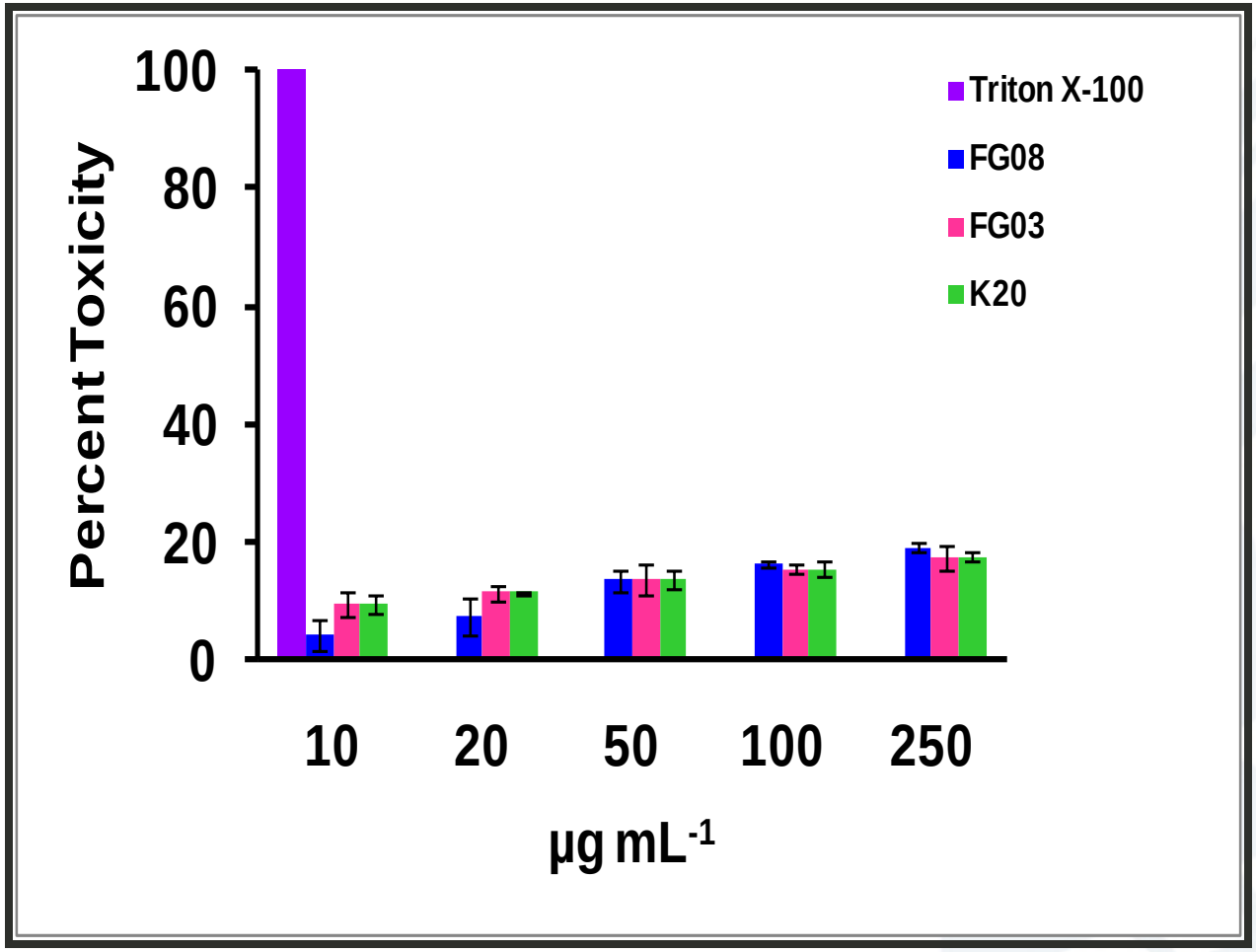
Over 1 Kg has been produced

Minimum Inhibitory Concentration

Microorganisms	[a]MIC µg mL^{-1}		
	FG08	FG03	K20
Bacteria			
Staphylococcus aureus 25923	62.5	nd	>250
E. coli ATCC25922	250	500	250
Filamentous Fungi			
Botrytis cinerea	31.3	31.3	31.3
Curvularia brachyspora	31.3	31.3	62.5
Pythium ultimum	15.6	31.3	62.5
Verticillium spp.	15.2	15.2	nd
Microdochium nivale	3.9	nd	3.9-7.8
Fusarium graminearum	7.8	7.8	7.8
Rhizopus stolonifer	31.3	[b]nd	62.5
Cladosporium cladosporioides	31.3	nd	nd
Fusarium oxysporum	7.8	nd	nd
Ulocladium spp.	7.8	nd	nd
Phoma spp.	31.3	nd	nd
Yeasts			
Candida albicans	31.3	62.5	31.3
Rhodotorula pilliminae	7.8	62.5	31.3

Cytotoxicity against C8161.9 cells

(human melanoma cells)



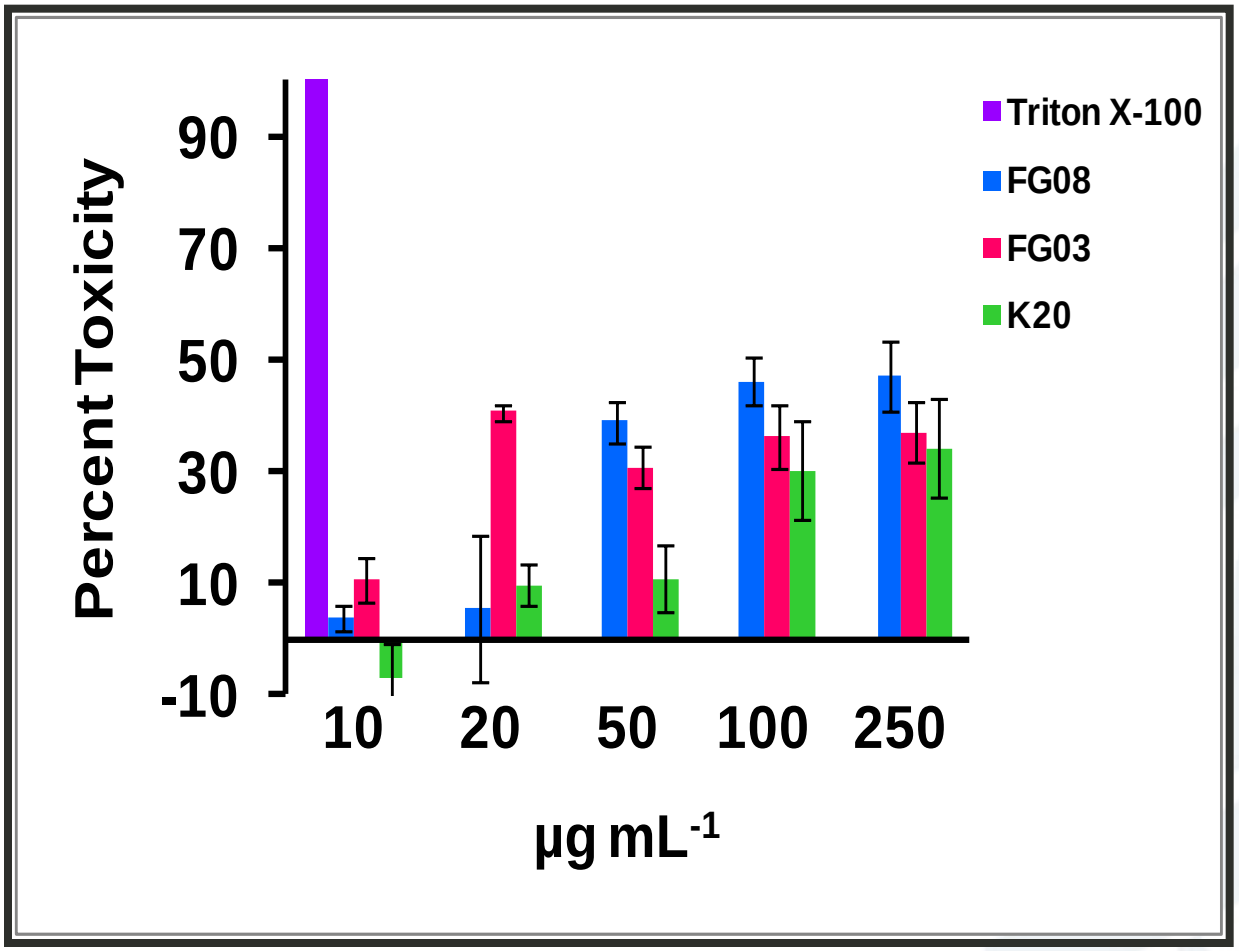
MTT assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

In collaboration with Kawasaki, Y.; Shrestha, S; Takemoto, J. Y.

Cytotoxicity against NIH3T3 cells

(mouse fibroblast)



MTT assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

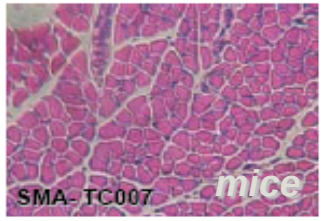
In collaboration with Kawasaki, Y.; Shrestha, S; Takemoto, J. Y.

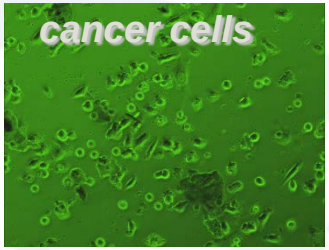
Novel Fungicide: K20

Specific against fungi

US and international patent pending

Low toxicity toward mammal



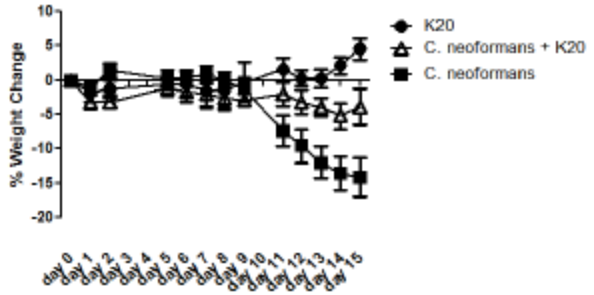


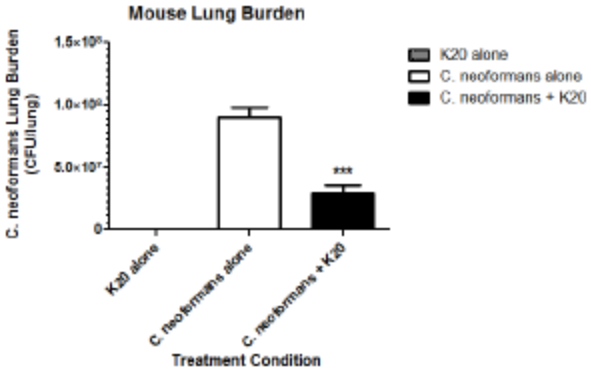
Effective in greenhouse and field

untreated

treated

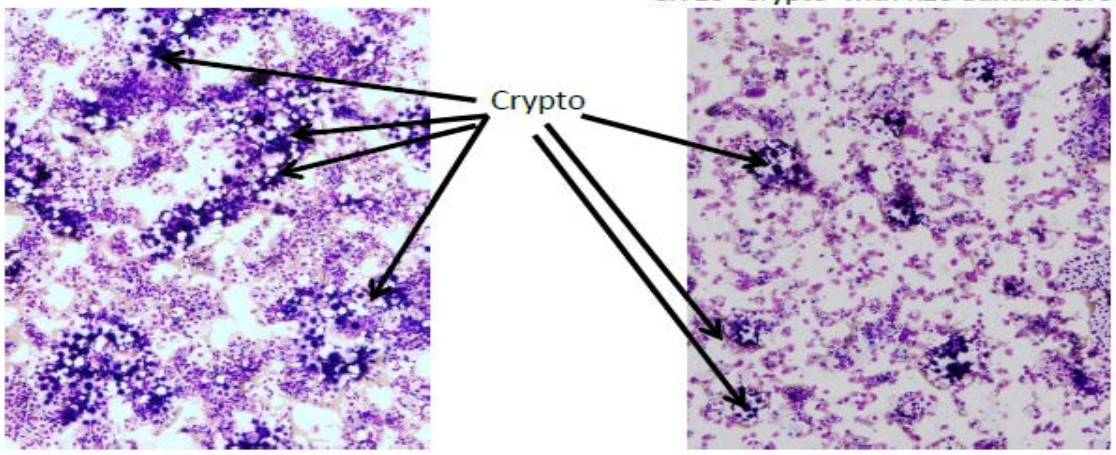
K20 Is Active Against *Cryptococcus neoformans*





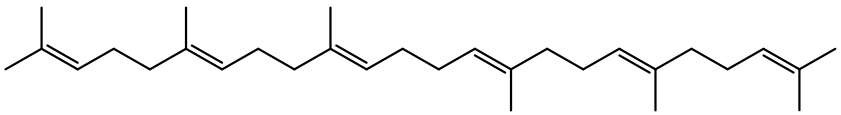
5X 10^3 Crypto ; no treatment

5X 10^3 Crypto with K20 administered once!



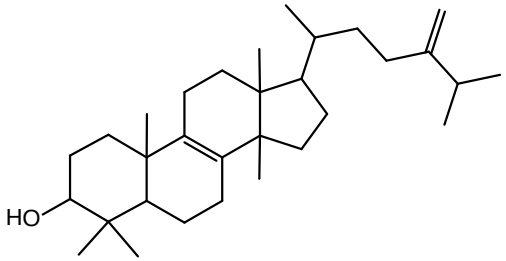
In collaboration with Prof. Nicole Meissner, Montana State University

The Ergosterol Biosynthesis



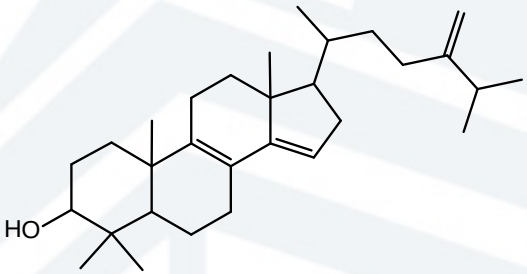
squalene-epoxidase

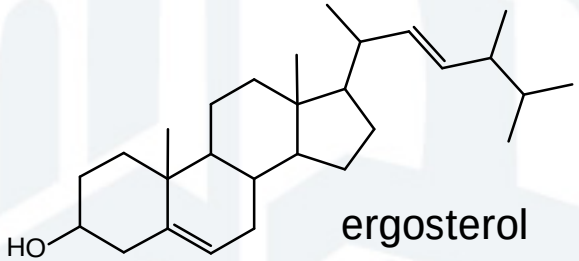
epoxisqualene-cyclase



C-14-demethylase

Azoles (triazoles, diazoles and thiazoles)





ergosterol

- Azoles inhibit demethylation
- Sterol Biosynthesis Inhibitors or **SBI** Fungicides

a critical component of fungal cell membranes

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