Antifungal Potential of Peptide Mimetics in a Mouse Model of Invasive Candidiasis

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Invasive Candidiasis: 4th common cause of hospital acquired infection in US

Available Therapy for Invasive Candidiasis

Antifungal drugs:

- Fluconazole
- Amphotericin-B
- Echinocandins

Limitations:

- Resistance
- Toxicity

Antimicrobial Peptides (AMPs)

Characteristics:

- Cationic & amphipathic in nature
- Broad-spectrum antimicrobials
- Little Resistance
- Low antigenicity

Limitations:

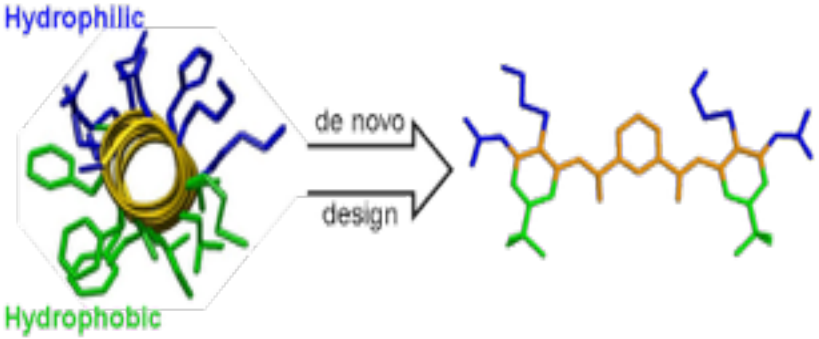
- Protease sensitive
- Expensive
- Often are inactivated by other proteins

Antimicrobial Peptide Mimetics

Designed and synthesized to mimic the action of AMPs in both structure and activity based on their cationic and amphipathic characteristics

Advantages:

- Broad spectrum activity
- Active against drug resistant strains
- Low resistance potential
- Not degradable by Proteases
- Facile synthesis and inexpensive manufacturing



Designed by chemists at Fox Chase Chemical Diversity Center

Published report of our lab:

Ryan LK et al. 2014. Antimicrobial Agents and Chemotherapy 58:3820-3827

- Peptide mimetics are effective against both planktonic and biofilm form of *Candida albicans*.
- Peptide mimetics are also effective for the topical treatment of oral candidiasis in mouse
- Low *in vivo* systemic toxicity upon oral gel treatment
- Failed to develop resistant strains of *Candida albicans*

Hypothesis

Safe

Non-Toxic

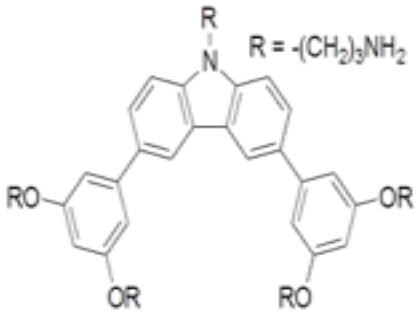
Peptide mimetics

Efficacious to treat invasive candidiasis

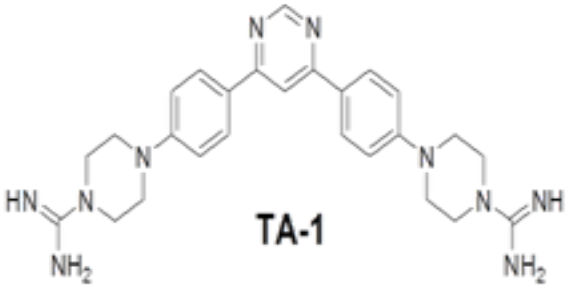
General Structure of Peptide Mimetic Compounds

Mimetics were designed based on the amphipathic and cationic structures of defensins

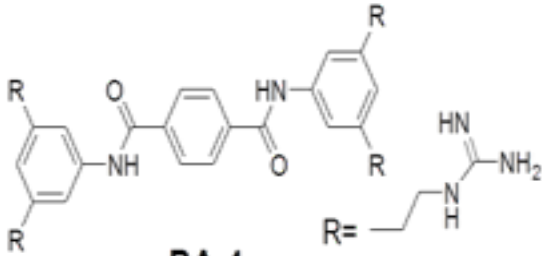
Three different series were designed: Tricyclic (TC), Triaryl (TA) and Bis-Amide (BA). eight promising leads were tested.



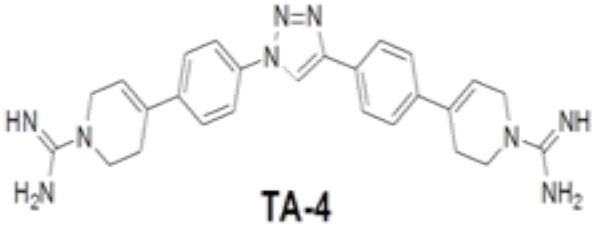
TC-1



TA-1



BA-4



TA-4

In vitro Antifungal Studies

Compound	**TC-1**	**TA-1**	**BA-4**	**TC-4**
Series	Tricyclic	Triaryl	Bis-Amide	Tricyclic
Activity against *C. albicans* (µg/ml)				
MIC	4	4	4	2
MFC	8	8	16	8
MIC + 50% Human serum	4	4	4	16
IC50	1.44	4.24	3.80	0.45
Other Yeast Species (MIC, µg/ml)				
C. tropicalis	2-4	4-8	8	4
C. parapsilosis	2	4-8	1	1
C. dubliniensis	4	8	32	16
C. glabrata	2	4	8	2
C. krusei	16	32	8	0.50
Cytotoxicity (µM)				
3T3	436	311	358	478
MTD (mg/kg free base)	20	10	10	10

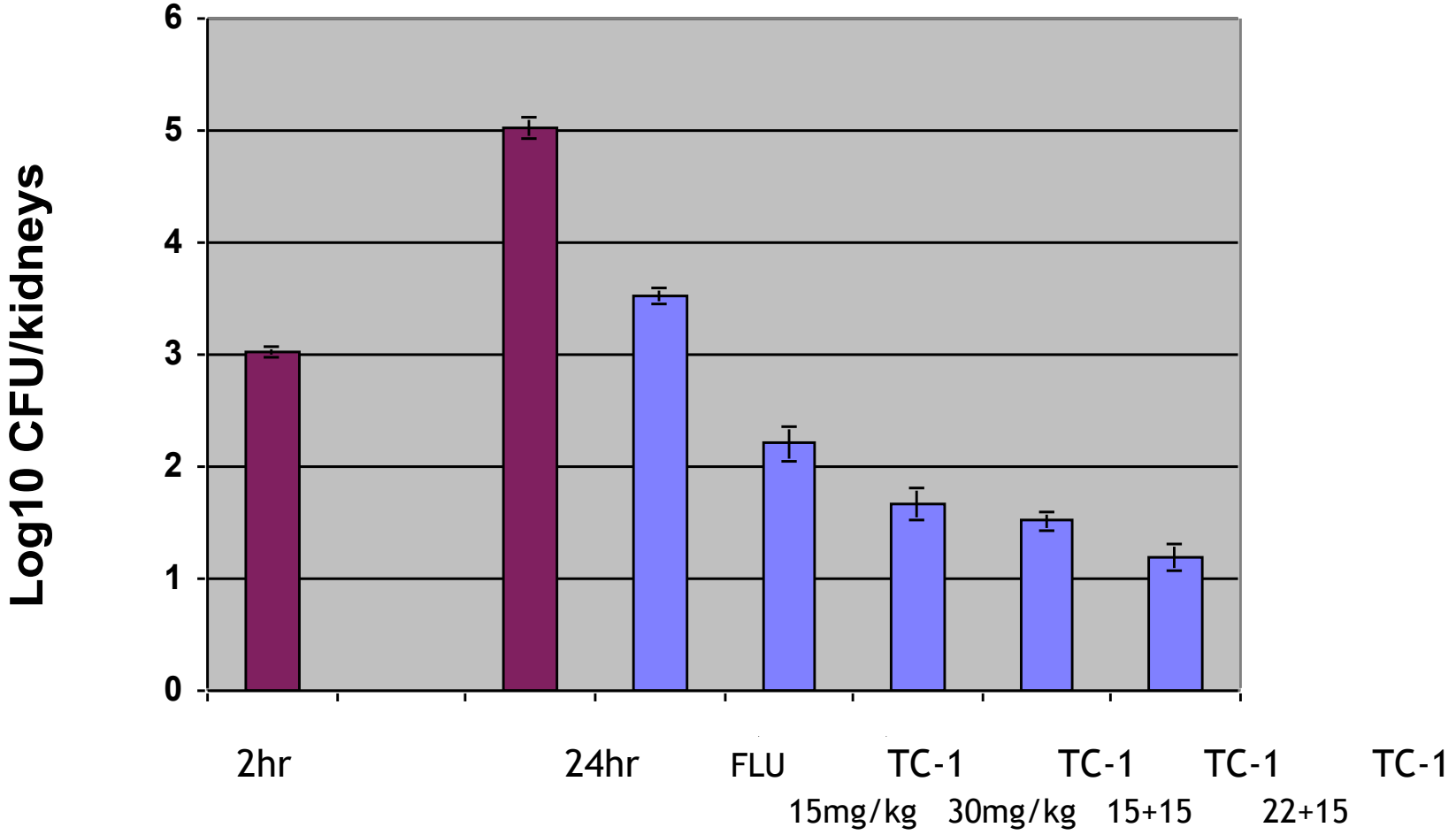
In Vitro Activity of TC-1 Against IC Clinical Isolates

Species	Strain	Resistance	MIC (μg/ml)	MFC(μg/ml)
C. albicans	SC5314	none	2	8
C. glabrata	TG-1	fluconazole	4	16
C. glabrata	TG-3	fluconazole	4	8
C. glabrata	TG-4	fluconazole	4	8
C. glabrata	TG-5	fluconazole	2	8
C. glabrata	TG-6	fluconazole	2	8
C. tropicalis	CT-2	fluconazole	4	16

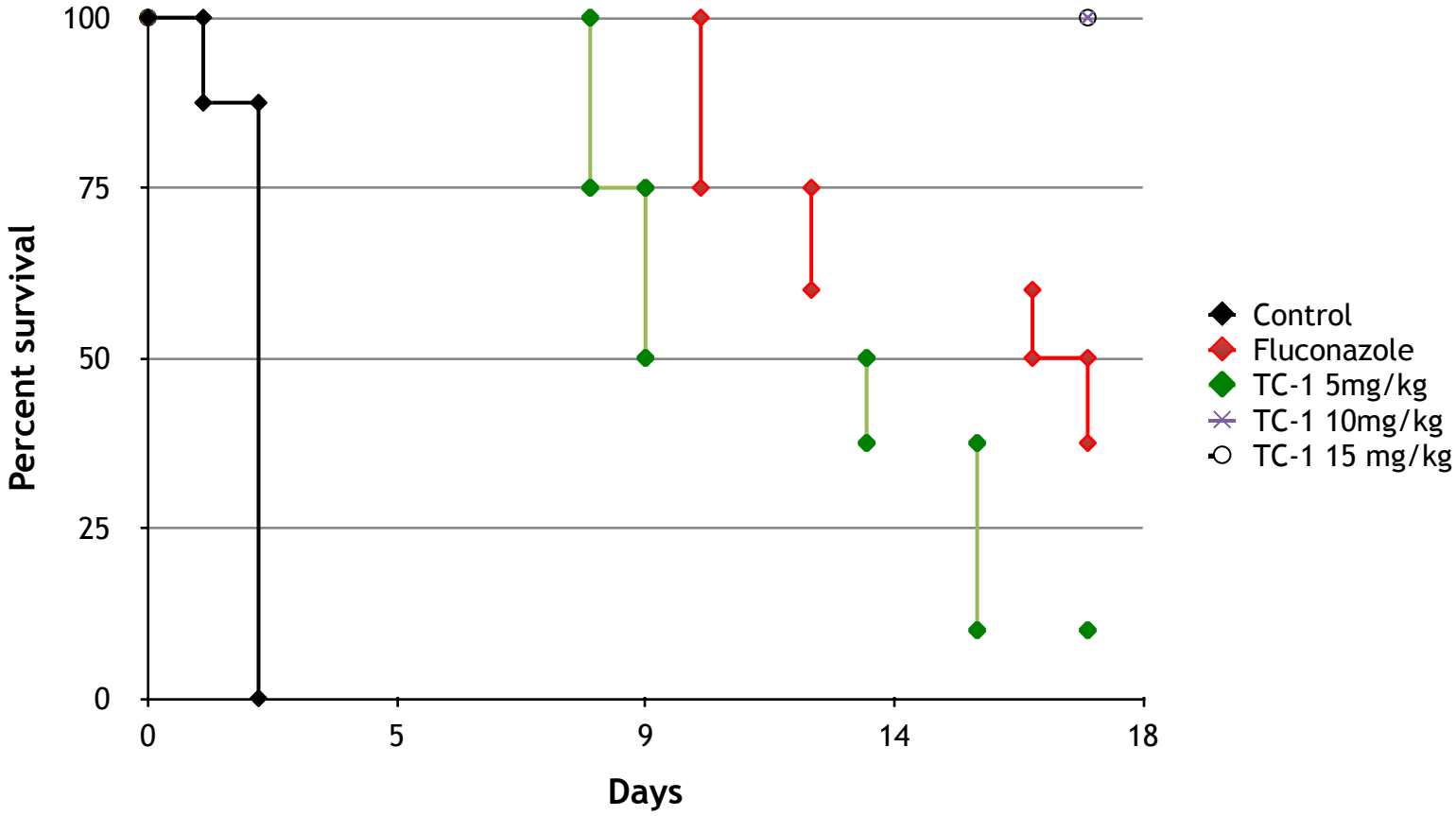
- Male CD-1 mice, 8 weeks old, were made neutropenic with i.p. injection of cylophosphamide(150 mg/kg in 10 mL/kg) at 4 and 1 day before inoculation
- Each animal was then inoculated by injecting 0.1 mL of 3.5 X 10^5 cfu / ml *C. albicans* in the tail vein
- Drugs were injected sub-cutaneously at 2 hour after inoculation

Experimental groups	Treatment	Group size	Time points
Infected control	*No treatment*	*5*	*2hr*
Infected control	*No treatment*	*5*	*24 hr*
Treatment 1	*High dose once (s.c.)*	*5*	*24 hr*
Treatment 2	*Medium dose once (s.c.)*	*5*	*24 hr*
Treatment 3	*Low dose once (s.c.)*	*5*	*24 hr*
Fluconazole	*20 mg/kg oral gavage*	*5*	*24 hr*
Vehicle	*(s.c.)*	*5*	*24 hr*

TC-1: *In vivo* Dose Response Study



TC-1 Survival Study

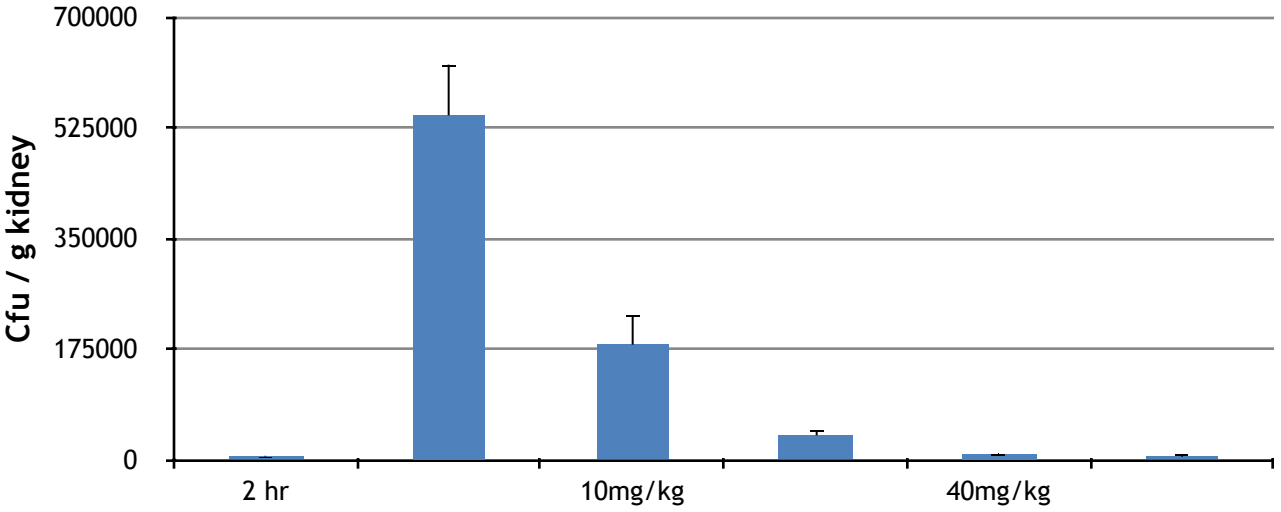


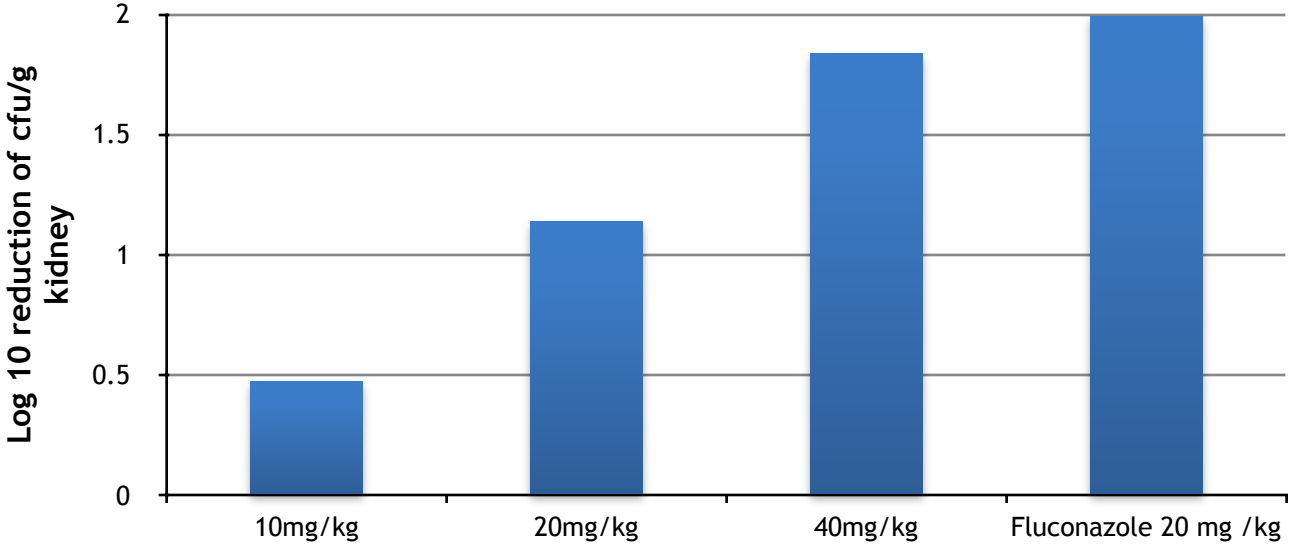
New Compounds Tested

Compound	Series
FC3785	Bis-amide
FC4812	Bi-aryl
FC4073	Bis-amide
FC4995	Bis-amide

Compounds	FC3785	FC4812	FC4073	FC4995
In vitro data				
Activity against C. albicans (µg/ml)				
MIC	*4*	*4*	*4*	*8*
MFC	*8*	*8*	*8*	*16*
MIC+50% Human Serum	*8*	*8*	*8*	*16*
MFC+50% Human Serum	*16*	*16*	*16*	*32*
Other Yeast Species (MIC, µg/ml)				
C. tropicalis	*2*	*8*	*4*	*4*
C. parapsilosis	*1*	*2*	*2*	*4*
C. dublinensis	*2*	*4*	*2*	*4*
C. glabrata	*4*	*4*	*4*	*8*
C. krusei	*2*	*4*	*2*	*4*
Cytotoxicity EC_{50} (µM)				
3T3	*891*	*>788.6*	*>1398.6*	*390*
HEPG2	*315.1*	*>788.6*	*>1398.6*	*249*
Hemolysis EC_{50} (µM)	*1453.5*	*>1577.3*	*1331*	*>1272*
In vivo data				
MTD (mg/kg free base)	*5*	*20*	*≥40*	*≥40*
Log_{10} reduction of kidney burden	*1.41*	*0.81*	*2.03*	*1.85*

FC4073: Dose Response Study





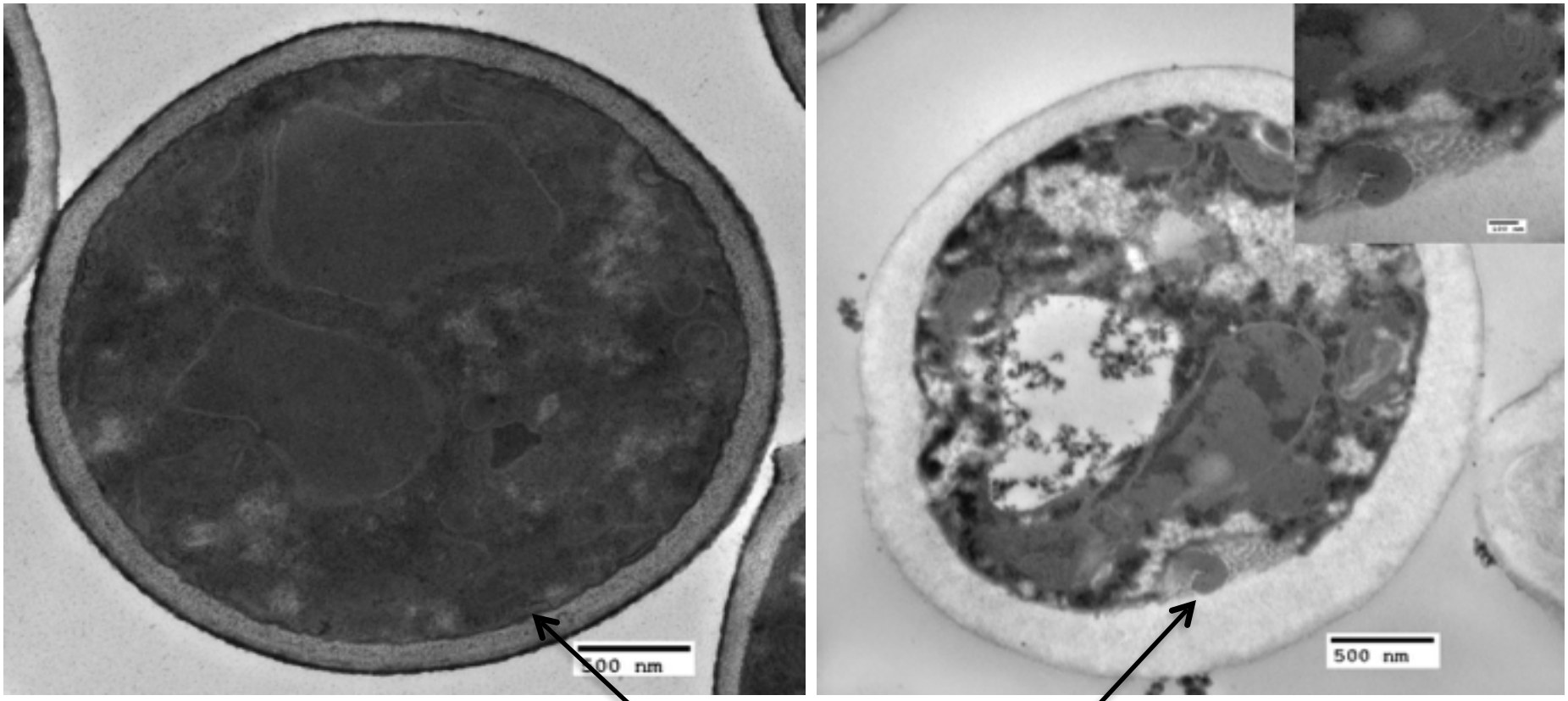
Summary
Mechanism of Action Studies

- Inhibition by cations (Mg^{++}, Ca^{++} and La^{+++}) show that peptide mimetics bind to the anionic surface head groups of the fungal membrane
- Mimetics rapidly form pores in the membrane as shown by propidium iodide incorporation
- Membrane is disrupted leading to cell death (EM and fluorescence microscopy)

Membrane Disruption

Control

TC-1



Plasma membrane

Summary and Conclusions

- We have identified a number of small molecule mimics of antimicrobial peptides that exhibit potent antifungal activity against *C. albicans in vitro*, and *in vivo* in a mouse model of invasive candidiasis

- Compounds are membrane active and action is very rapid, so no resistance develops

- These compounds show great potential for development as effective antifungal drugs to treat both topical and systemic candidiasis

Acknowledgement

Diamond Lab:
Gill Diamond, Ph.D.
Lorenzo Menzel, Ph.D.
Kristina DiFranco, Ph.D.
David Brice
William Ruddick

Division of Infectious Diseases and Global Medicine, Department of Medicine, University of Florida College of Medicine:
Lisa Kathleen Ryan, Ph.D.
Kartikeya Cherabuddi, MD

UF Health SHANDS Clinical Laboratory
Beth Fisher
Alyssa Lewis

Fox Chase Chemical Diversity Center:
Richard W. Scott, Ph.D.
Katie Freeman, Ph.D.
Damian Weaver, Ph.D.
Jeff Pelletier, Ph.D.

Cellceutix, Inc.
Leo Ehrlich, CPA
Krishna Menon, VMD, Ph.D.

Funding:
National Institutes of Health (NIAID)
R44AI106270

Technical assistance from Ricerca, Inc.