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In Vivo Imaging of the Activity of Host Defense Peptide Mimetics in a Mouse Model of Invasive Candidiasis

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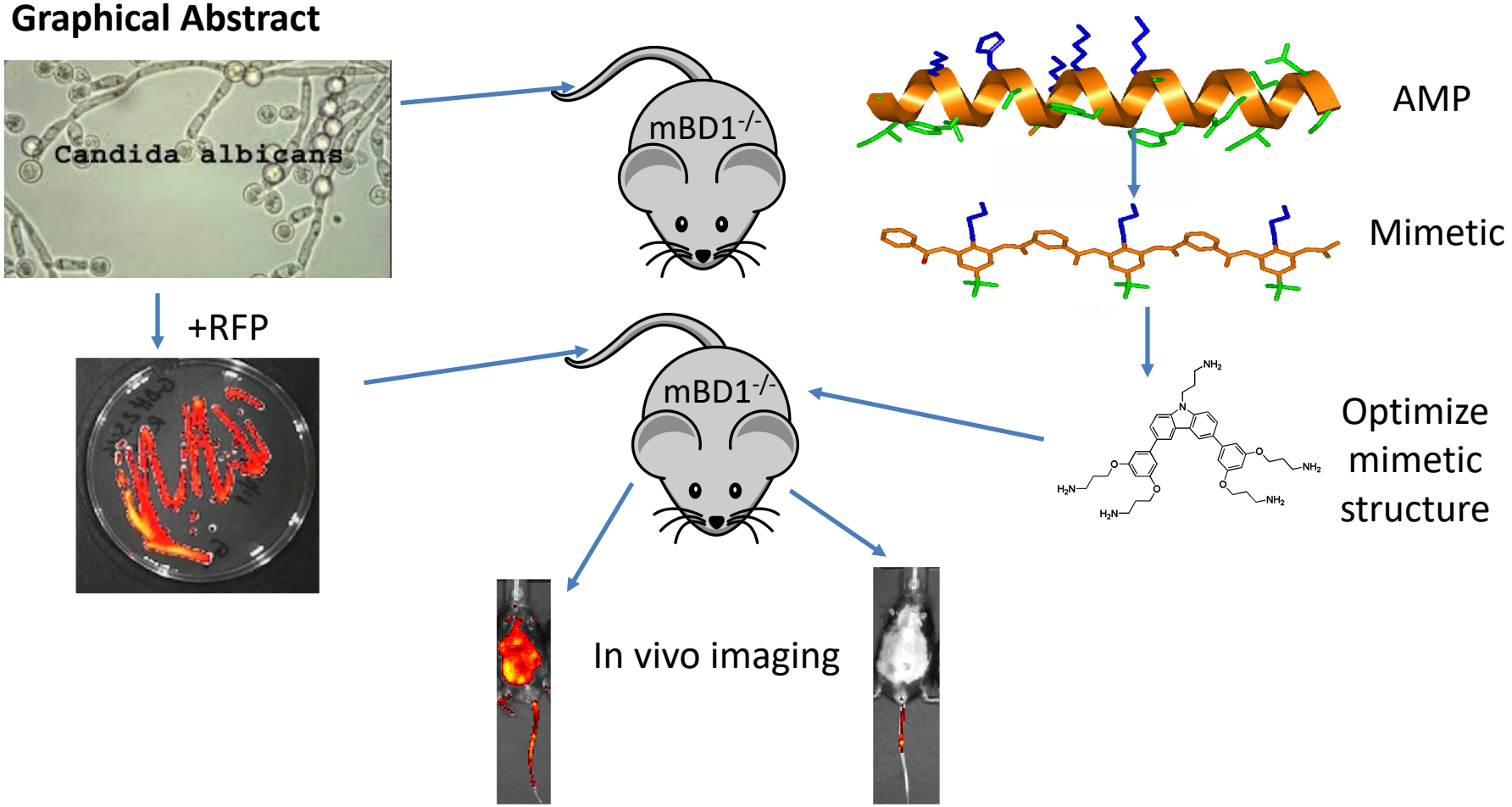
In Vivo Imaging of the Activity of Host Defense Peptide Mimetics in a Mouse Model of Invasive Candidiasis

Graphical Abstract



Systemic fungal infections are increasingly common, especially in immune compromised patients. Even with newly developed drugs, there remain issues of limited spectrum, side effects, and the development of resistance. Host defense peptides (HDPs) have been examined recently for their utility as therapeutic antifungals, especially due to the low levels of resistance that develop. Unfortunately, the peptides exhibit poor pharmacologic properties in vivo. We have demonstrated the potent activity of nonpeptidic compounds that mimic HDPs in both structure and function against clinical strains of *Candida albicans* associated with oral and invasive candidiasis in mouse models. However, to test numerous compounds *in vivo* requires large numbers of mice, with multiple time points, and requires immunosuppression of the mice using cyclophosphamide, which can influence pharmacological parameters. We have identified a strain of mouse that develops invasive candidiasis without the need for immunosuppressive drugs. When we infect these mice with a strain of *C. albicans* that constitutively expresses Red Fluorescent Protein, we can quantify the infection in real time by in vivo imaging. We can further observe the reduction in fluorescence in infected mice after treatment with an HDP mimetic. Together our results demonstrate a novel in vivo method for screening new antifungal drugs.

Keywords: Antimicrobial peptide; antifungal; Candida; peptidomimetics

Introduction- Antimicrobial peptides

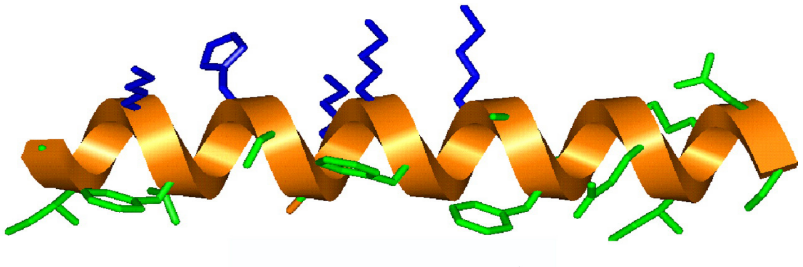
- Short, generally cationic, broad-spectrum antimicrobial proteins
- Found at mucosal surfaces
  - Skin secretions in fish and amphibians
  - Oral cavity, trachea, small intestine, female reproductive tract in mammals
- Found in myeloid cells
  - Neutrophils, alveolar macrophages

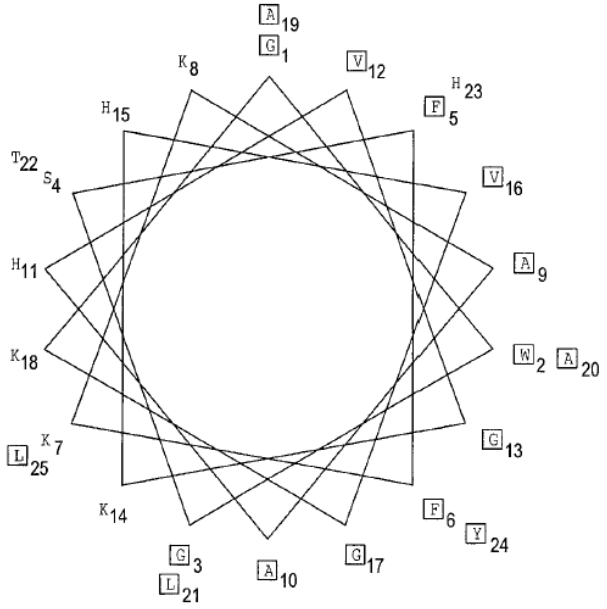
Types of AMPs

- Linear
  - Amphipathic α-helical
- Cysteine-rich
  - β-sheet
- Peptides with specific amino acids
  - Rich in His, Pro or Trp

Linear peptides

- Magainin (frog skin)
- Pleurocidin (fish skin)
- Cecropin (insect)
- Protegrin (pig leukocytes)
- LL-37 (human cells)

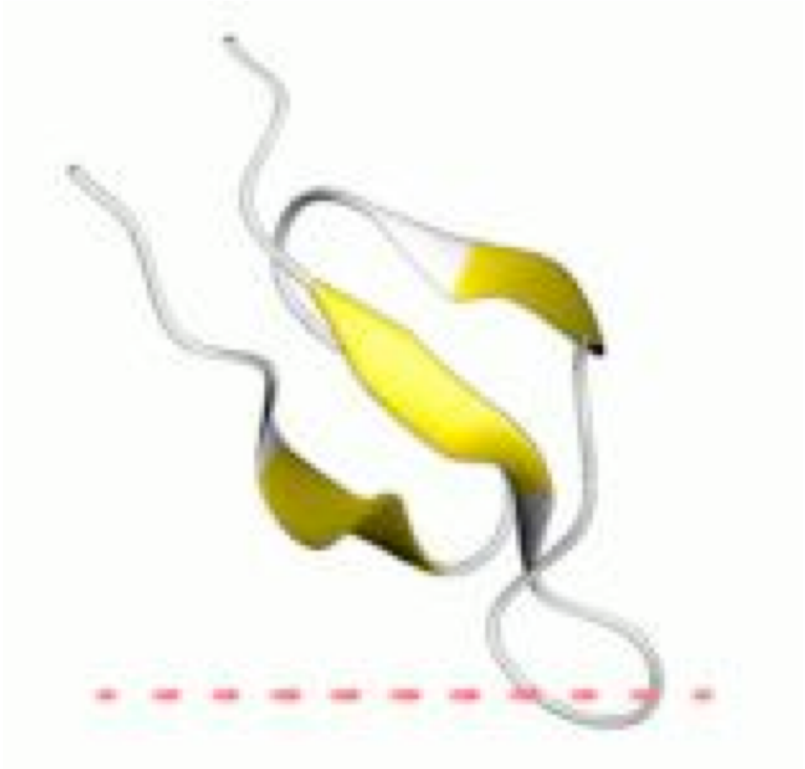




Form cationic amphipathic α-helices

Cysteine-rich peptides

- α-defensins
  - PMNs, small intestine
- β-defensins
  - Epithelial cells, some blood cells
- θ-defensins
  - Rhesus monkey PMNs



Natural Roles of AMPs

- Antimicrobial defense of surfaces
  - magainins on amphibian skin
  - β-defensins on mammalian epithelium
- Oxygen-independent antimicrobial activity of phagocytic cells
  - α-defensins in PMNs
- Chemotactic agents for innate immune defense cells
  - β-defensins, LL-37

Antimicrobial Peptides as Therapeutics

GOOD

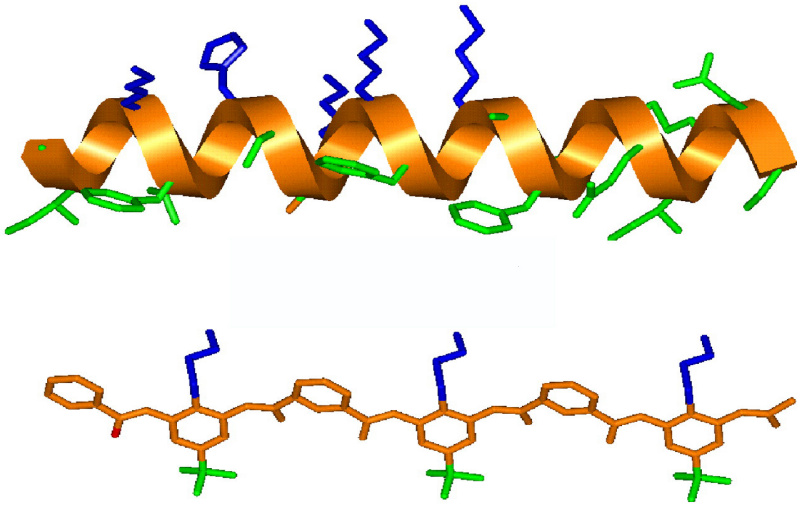
- Naturally occurring
- Broad-spectrum antimicrobials
- Little resistance
- Low antigenicity

BAD

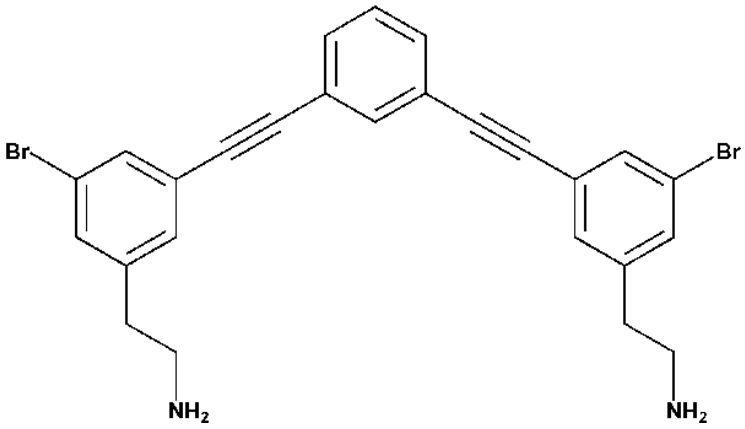
- Protease sensitive
- Expensive to produce and purify
- Often are inactivated by other proteins

Peptide Mimetics

- Structurally similar to active portion of AMP
  - Cationic, amphipathic
- Protease resistant
- Inexpensive to produce



Activity of an AMP mimetic against oral pathogens



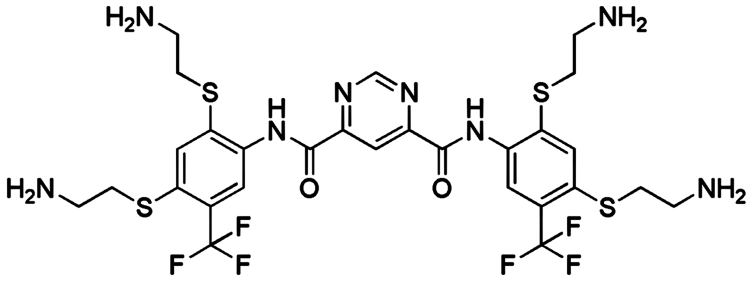
Bacterium (strain)	MIC, µg/ml	MBC, µg/ml
S. mutans (ATCC33402)	0.5-1	1.25
S. mutans (isolate N32)	0.5	1.25
S. mutans (isolate N43)	0.5	1.25
S. aureus (ATCC 27660)	0.25-0.5	0.8
P. gingivalis (ATCC 53978)	2.5	2.5
A. actinomycetemcomitans (CU1000)	0.4	1.5
A. viscosus	0.8	1.5

Beckloff et al., Antimicrobial Agents Chemother., 51:4125, 2007

Hypothesis

- Antimicrobial peptide mimetics could be useful therapeutic agents to treat fungal infections

Activity of a peptide mimetic against Candida spp. in vitro



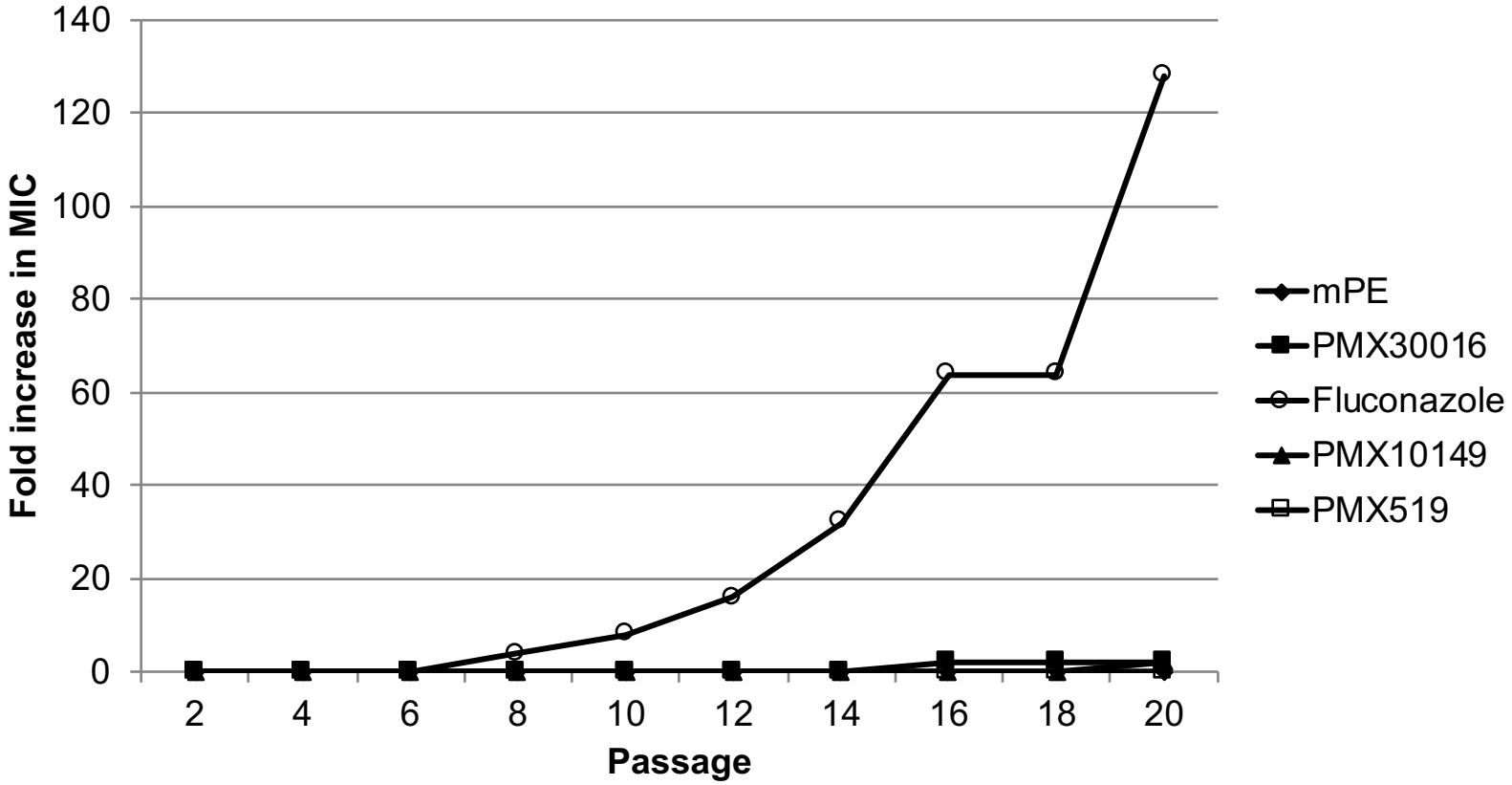
Species	MIC, µg/ml
C. albicans	4-8
C. tropicalis	4
C. parapsilosis	8
C. glabrata	16
C. dubliniensis	8
C. krusei	8

Screening for New Compounds

Compound	Anti-*C. albicans* GDH2346 (μg/ml)		Cytotoxicity EC50 (μM)			Anti-bacterial; commensals MIC (μg/ml)		MTD (mg/kg)
	IC50	MIC	NIH3T3	HepG2	OKF6/ TERT	*Streptococcus salivarius*	*Actinomyces viscosus*	
PMX70004	4.88	4-8	52	31	68	16	32	10
PMX519	4.93	4-8	439	>1000	>1000	>64	>64	17
PMX1408	4.24	4	311	453	466	16	4	< 2.5
PMX1502	1.44	4	436	885	766	>64	>64	20
PMX1570	1.09	2	108	310	371	8	4	10
PMX1576	1.03	2	149	288	502	8	4	5
PMX1591	2.2	2	461	904	ND	32	8	ND
PMX1625	2.08	2	523	723	718	64	16	15

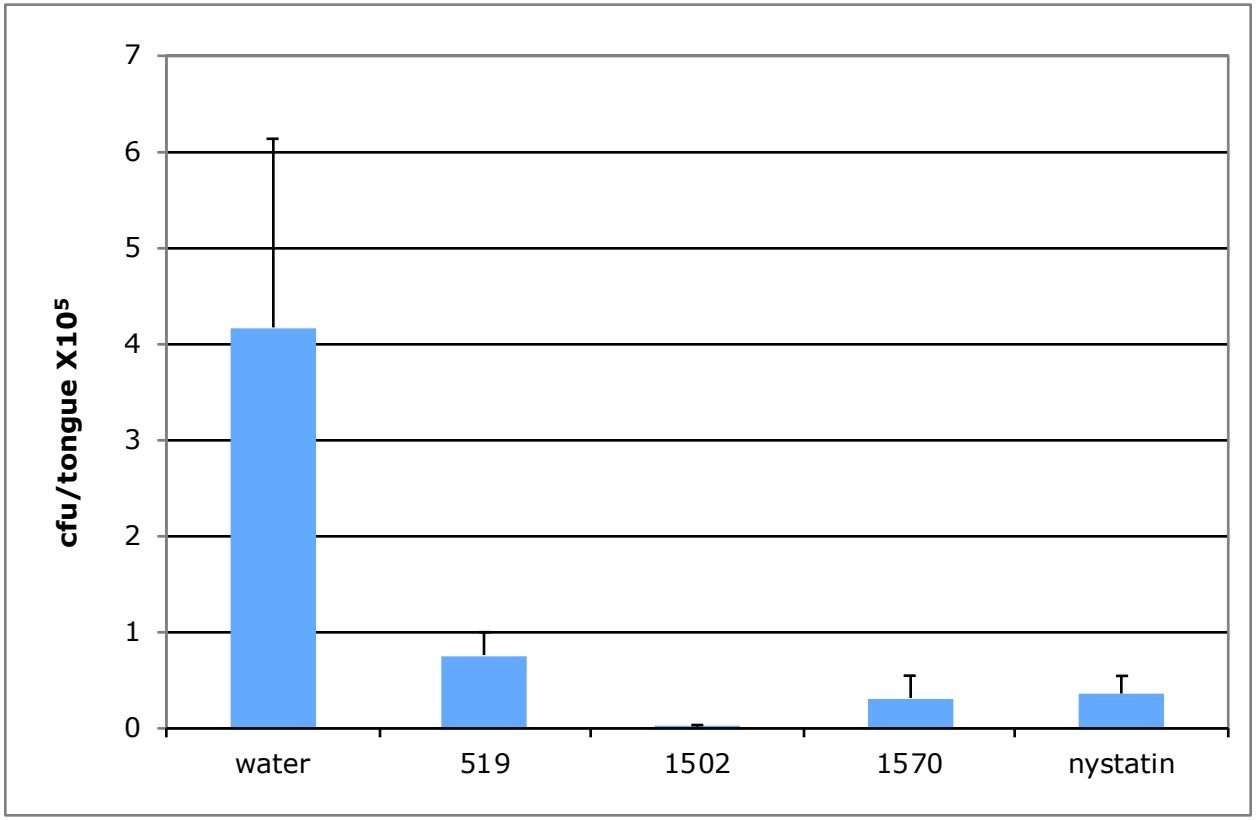
Ryan et al., Antimicrob. Agents Chemother. 58:3820, 2014

No Development of Resistance



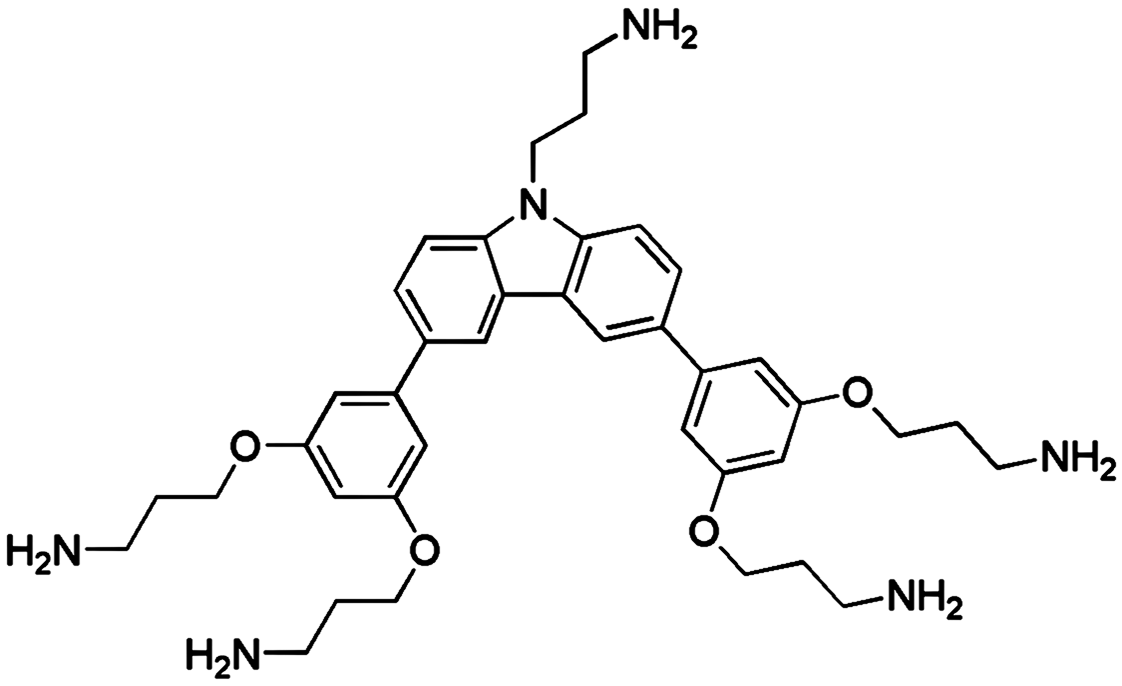
Hua et al., Mol. Oral Microbiol. 25: 418, 2010

Peptide mimetics are active in a mouse model of oral candidiasis

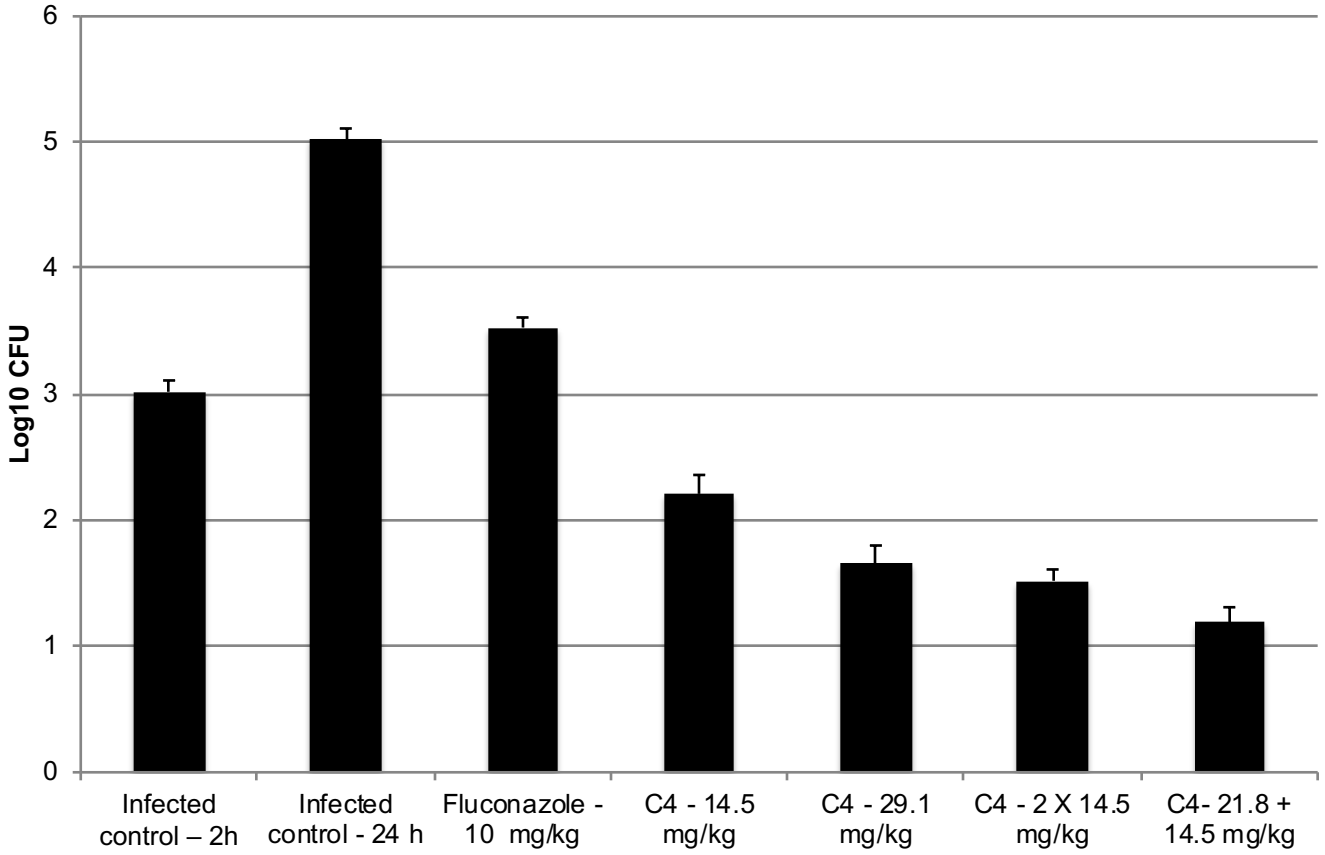


Ryan et al., Antimicrob. Agents Chemother. 58:3820, 2014

Lead compound, PMX1502 (also called C4)

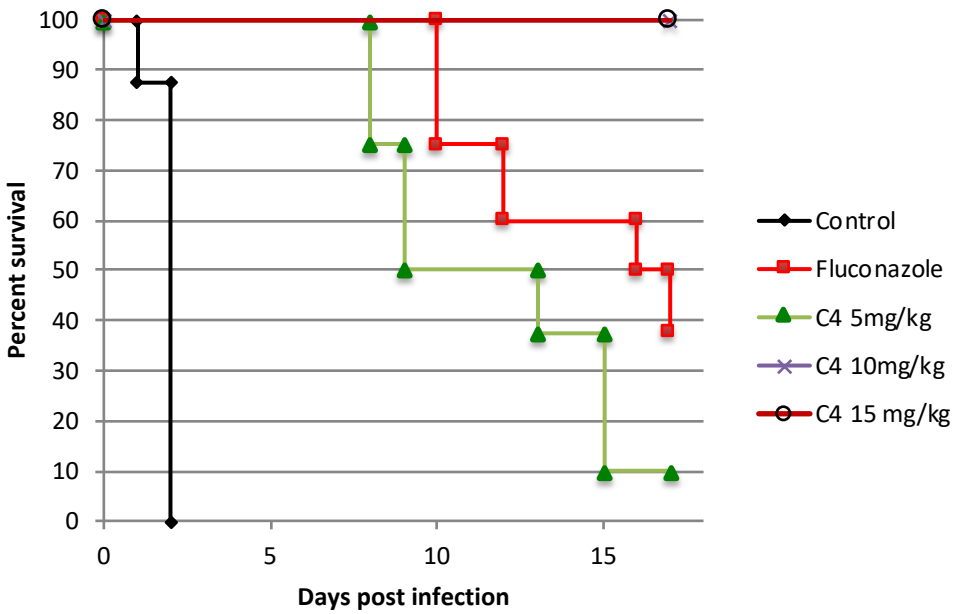


Activity in a model of invasive candidiasis-kidney burden



Menzel et al., Sci. Rep. 7:4353, 2017

Disseminated Candidiasis Model; Survival Study

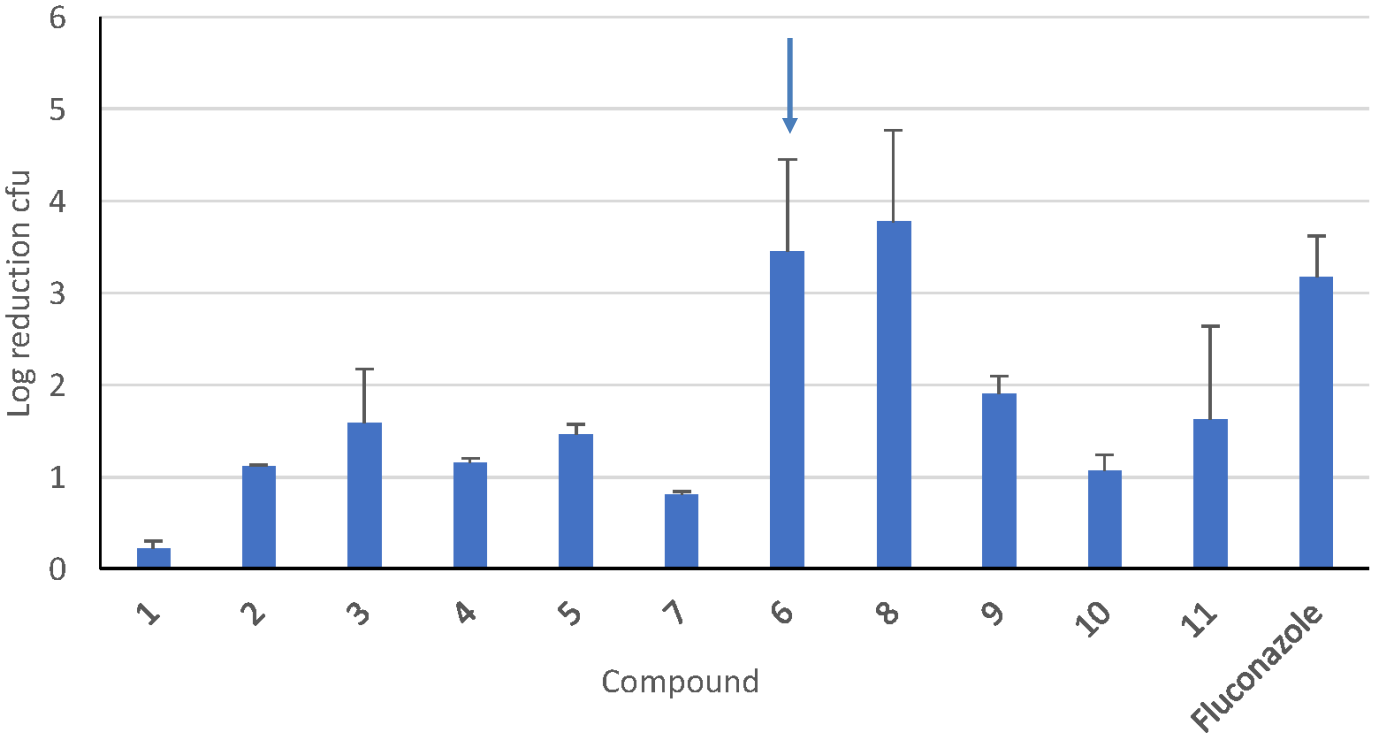


100% survival in 10 and 15 mg/kg C4 groups, no overt toxicity

40% survival in the fluconazole group

Menzel et al., Sci. Rep. 7:4353, 2017

Screening of new compounds in vivo

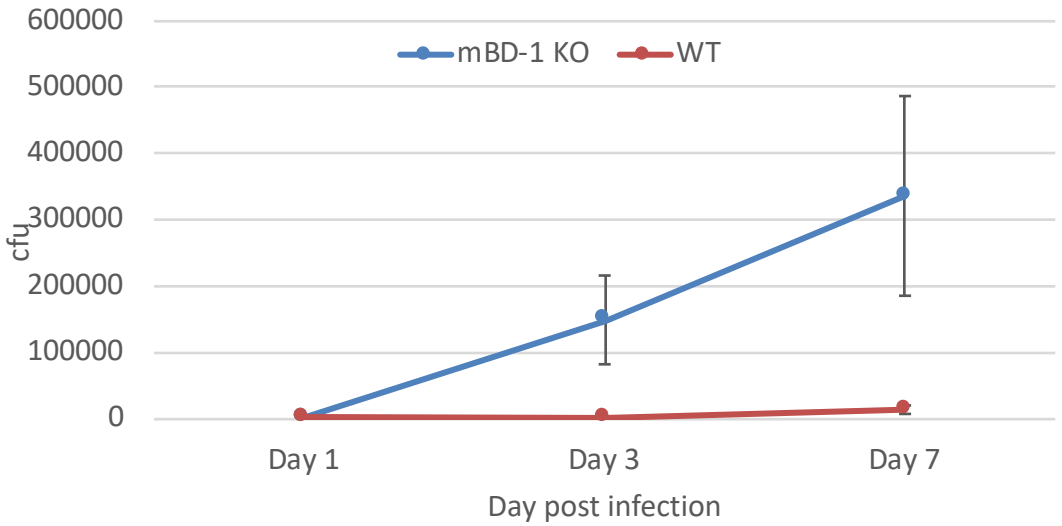


Chowdhury et al, J. Fungi, 4:30, 2018

Results and discussion

1. AMP mimetics can be designed and screened to obtain highly active antifungal drugs that act in vivo to treat oral and invasive fungal infections.
2. Screening large numbers of mimetics in vivo requires large numbers of mice, especially for dose response and time course studies.
3. We wished to develop a mouse model of invasive candidiasis that would require fewer mice, to allow for more efficient screening of AMP mimetic activity against fungal pathogens in vivo.

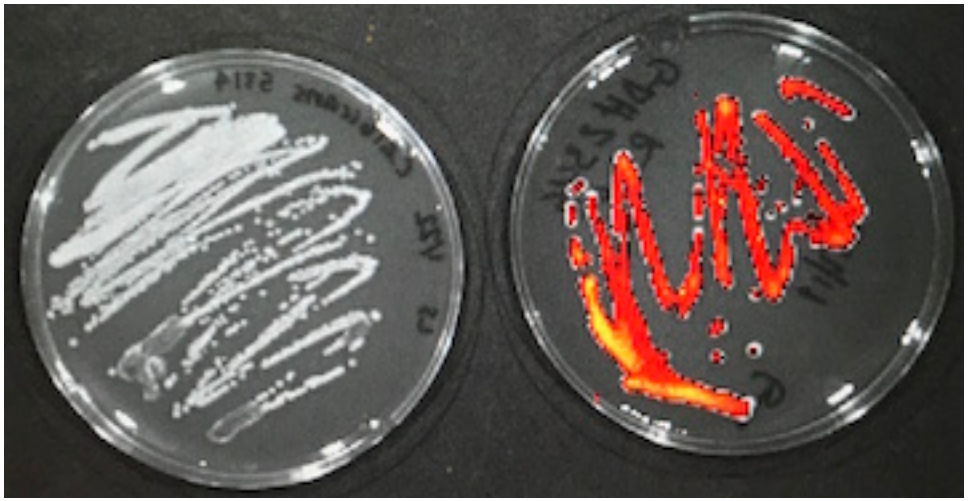
1. **Development of a non-immunosuppressed mouse model of invasive candidiasis**
   a) **Used C57Bl/6 mice deficient in mouse β-defensin 1 (mBD1$^{-/-}$)**
   b) **Injected 5x10^{5}cfu *C. albicans* IV into tail vein**
   c) **Quantified viable cfu in kidneys**



mBD1-/- mice are a good strain to test antifungal drugs without the need for immunosuppressive pre-treatment

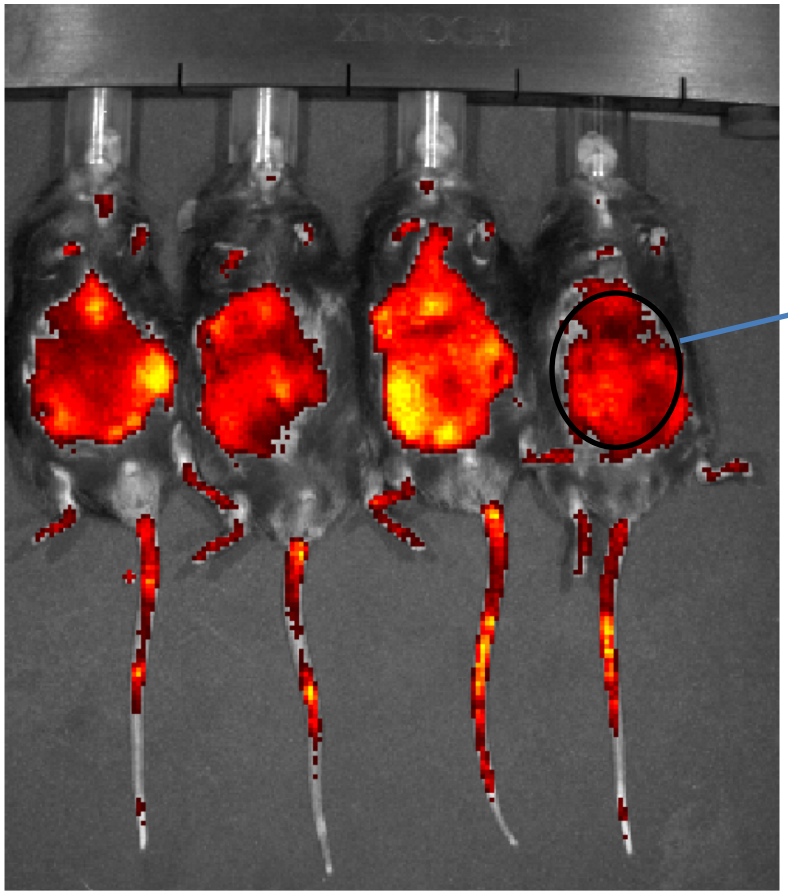
2. Develop a fluorescent strain of *C. albicans*

C. albicans GDH2346 *C. albicans* GDH2346-RFP



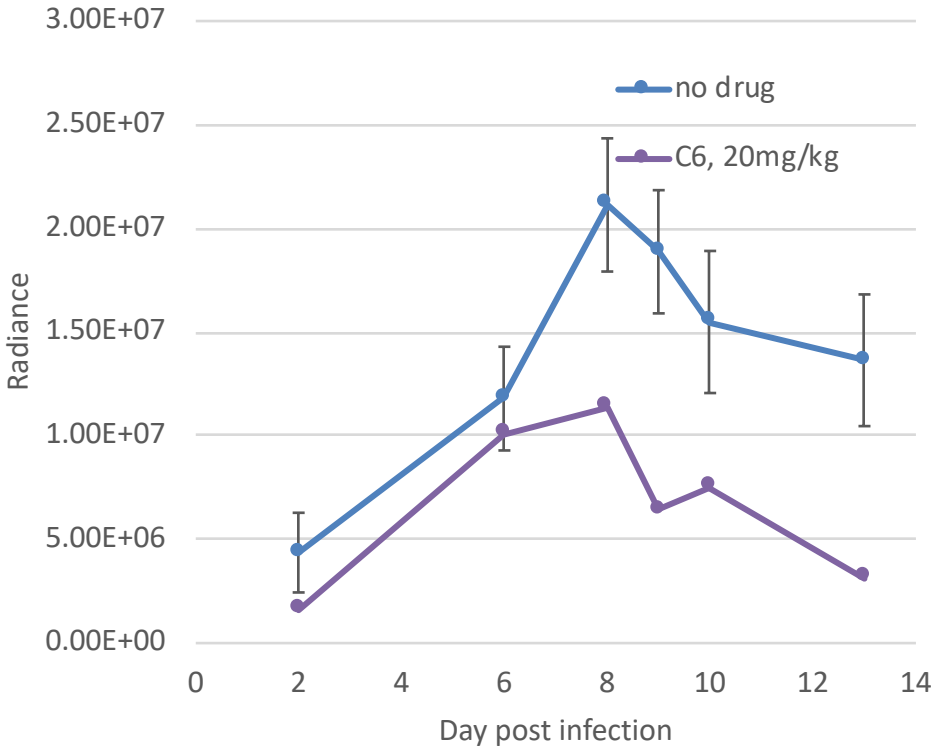
Can readily visualize and quantify fluorescence of Candida in the Xenogen in vivo imaging system (IVIS)

3. Infect with *C. albicans*-RFP ($5x10^4$cfu). Inject AMP mimetic (C6) 2 hours post infection. Image mice daily



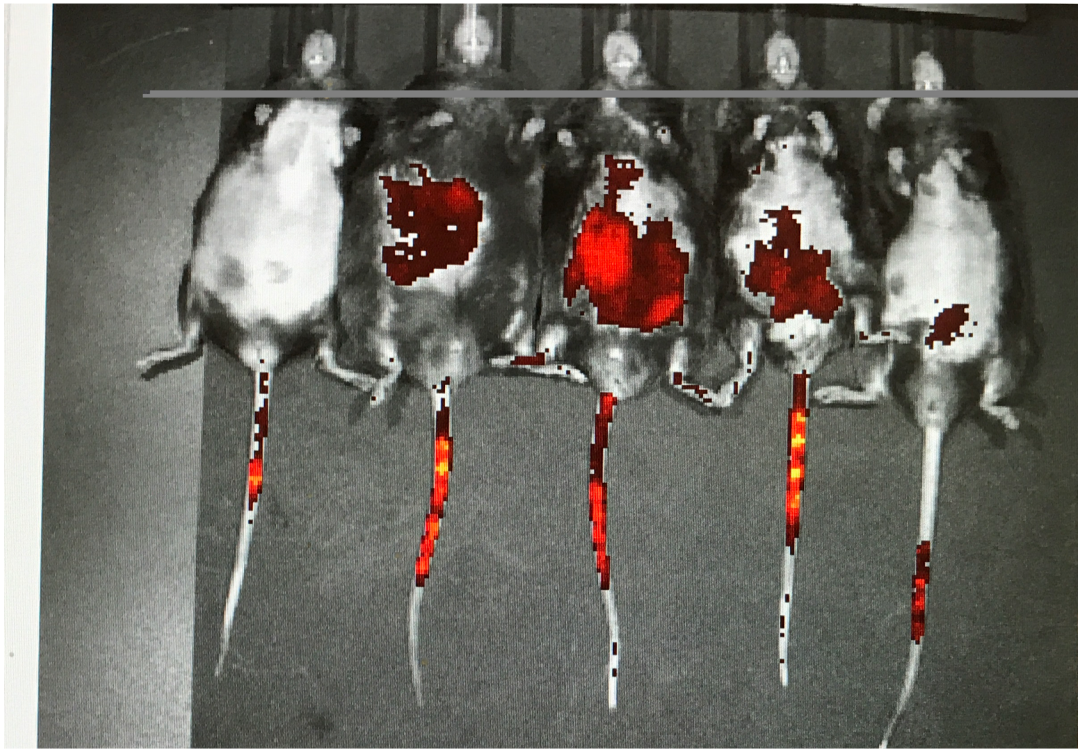
Quantify region of interest

4. Quantify fluorescence daily by IVIS



Compound C6 inhibits growth of *C. albicans* over time

5. Dose response (day 10 post infection)



No infection | 0mg/kg | 5 | 10 | 20

Compound C6 inhibits infection in a dose-dependent manner

Conclusions

1. We have developed a novel, non-lethal infection model for invasive candidiasis in mice.
2. We have used a fluorescent strain of *C. albicans* to allow observation and quantification of infection in real time.
3. We have shown that antimicrobial peptide mimetics can be tested in this model to assist in more efficient and rapid screening of novel antifungal agents.
4. This will hopefully lead to more efficient in vivo screening of antifungal drugs, with the use of less mice.

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UF Clinical and Translational Science Institute
UNIVERSITY of FLORIDA