Published in final edited form as:

Mol Oral Microbiol. 2010 December 1; 25(6): 418–425. doi:10.1111/j.2041-1014.2010.00590.x.

Activity of Antimicrobial Peptide Mimetics in the Oral Cavity: I. Activity Against Biofilms of *Candida albicans*

Jianyuan Hua¹, Radha Yamarthy¹, Shaina Felsenstein¹, Richard W. Scott², Kenneth Markowitz¹, and Gill Diamond^{1,*}

¹Department of Oral Biology, UMDNJ-New Jersey Dental School, Newark, NJ 07101

²PolyMedix, Inc., Radnor, PA

Summary

Naturally occurring antimicrobial peptides hold promise as therapeutic agents against oral pathogens such as *Candida albicans*, however numerous difficulties have slowed their development. Synthetic, non-peptidic analogs that mimic the properties of these peptides have many advantages and exhibit potent, selective antimicrobial activity. Several series of mimetics (MW <1,000) were developed and screened against oral *Candida* strains as a proof-of-principle for their antifungal properties. One phenylalkyne and several arylamide compounds with reduced mammalian cytotoxicities were found to be active against *C. albicans*. These compounds demonstrated rapid fungicidal activity in liquid culture even in the presence of saliva, and demonstrated synergy with standard antifungal agents. When assayed against biofilms grown on denture acrylic, the compounds exhibited potent fungicidal activity as measured by metabolic and fluorescent viability assays. Repeated passages in sub-MIC levels did not lead to resistant *Candida* in contrast to fluconazole. Our results demonstrate the proof-of principle for the use of these compounds as anti-*Candida* agents, and their further testing is warranted as novel anti-*Candida* therapies.

Keywords

antifungal; denture; fungicide; defensin; resistance

Introduction

Oral infections with *Candida*, oropharyngeal candidiasis (OPC), were observed in 90% of patients undergoing chemotherapy for acute leukemia (Rodu *et al.*, 1988), and 95% of patients with HIV (Dupont *et al.*, 1992). Although the introduction of highly active anti-retroviral therapy has reduced these numbers in HIV patients, the occurrence is still very high. In denture stomatitis, which commonly affects edentulous individuals (Webb *et al.*, 1998), *Candida* grows as a biofilm on the bioprosthetic materials used to make dentures (Edgerton *et al.*, 1993), and is highly resistant to standard antifungal agents (Chandra *et al.*, 2001). Treatment of candidiasis is either with topical antifungal agents such as Nystatin, or with systemic agents, including azoles, such as fluconazole or itraconazole, or echinocandins, such as caspofungin. With the recurrence of OPC in HIV patients, long-term treatments have led to a significant rise in antifungal-resistant organisms (for review, see (Ghannoum and Rice, 1999)).

*Correspondence: Dr. Gill Diamond, Department of Oral Biology, UMDNJ-New Jersey Dental School, 185 South Orange Ave., Newark, NJ 07103, USA, Tel: 973-972-3324, Fax: 973-972-0045, gdiamond@umdnj.edu.

With the initial discovery of naturally occurring, broad-spectrum antimicrobial peptides (AMPs) such as defensins and magainins (reviewed in (Diamond *et al.*, 2009)), activity against *C. albicans* was examined as a potential alternative to standard antifungal treatments. AMPs exhibit potent in vitro anti-Candidal activities under normal conditions (Selsted *et al.*, 1985, Benincasa *et al.*, 2006, Zasloff, 1987). Examination of human saliva also identified naturally occurring peptides known as histatins with anti-Candidal activity in vitro, (Oppenheim *et al.*, 1988), and synergy with antifungals such as azoles (Wakabayashi *et al.*, 1996). Synthetic peptides also exhibit potent activity against *Candida* species (Burrows *et al.*, 2006, Nikawa *et al.*, 2004)), and using a mouse model of candidiasis, peptides derived from lactoferrin exhibited activity in vivo (Takakura *et al.*, 2003), suggesting that molecules derived from AMPs would be useful drugs to treat these infections. However, *Candida* demonstrate an innate immune evasion strategy of proteolytic cleavage of AMPs (Meiller *et al.*, 2009).

Given their very broad specificity, amphiphilic AMPs appear to be ideal therapeutic agents. However, significant pharmaceutical issues, including poor tissue distribution, systemic toxicity and difficulty and expense of manufacturing, have severely hampered clinical progress. To address this problem, researchers have developed different types of small molecule peptide mimetics. These include peptoids, β -peptides, arylamide oligomers and phenylene ethynylenes (reviewed in (Rotem and Mor, 2008)). We have recently described a series of non-peptidic analogues that have many advantages over peptides because of their small size, which increases stability and enhances tissue distribution, and ability to fine-tune their physical properties for optimization of potency and safety (reviewed in (Som *et al.*, 2008)). These include the phenylethynylene derivative, mPE (PMX70004), which exhibits broad-spectrum antimicrobial activity against oral pathogens (Beckloff *et al.*, 2007), including three different strains of *C. albicans* and five non-*albicans* species (*C. glabrata*, *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*). Two other classes, based on arylamide and arylurea scaffolds also demonstrate broad-spectrum antimicrobial activity (Liu *et al.*, 2004, Tang *et al.*, 2005, Tang *et al.*, 2006). Together, they represent a class of AMP mimetics that exhibit the activity of AMPs, without the protease sensitivity and expense of the peptides. To assess their potential as antifungal therapies for oral *Candida* infections, we examined as a proof-of-principle the antifungal activities of representatives of the three classes of mimetics on *C. albicans*, both in planktonic states and in biofilms on denture material.

Materials and Methods

Yeast Strain

Candida albicans ATCC 90028 (obtained from the laboratory of Dr David Perlin, PHR/UMDNJ) were used for all assays and are cultured on YPD agar (1% yeast extract, 2% peptone, 2% dextrose, pH 5.7) at 37°C. For liquid assays, single colonies were dispersed in RPMI-1640 (Mediatech, Inc.) with MOPS, pH 7.0 at a concentration of 2.5×10^6 CFU/ml.

Peptide Mimetic Compounds

Twelve different peptide mimetic compounds developed by PolyMedix, Inc. were tested in antimicrobial screens (MIC assay). Further comprehensive assays were performed on the following four compounds, mPE (PMX70004), PMX30016, PMX000519, PMX10149 (for structures, see online supplemental figure 1), all of which exhibited MIC values below 8 μ g/ml. All compounds were dissolved in DMSO (Sigma) at the stock concentration of 20mg/ml, and stored at -20°C.

MIC assay

Assays were carried out in 96-well plates using the NCCLS method as previously described (Beckloff et al., 2007). Mimetic compounds were diluted in 50µl RPMI/MOPS in a 96-well plate (Tissue culture treated, Falcon). 50µl suspensions of *Candida* were added to each well, and the plate was then incubated at 37°C in a humidified chamber for a period of 24 hours. MIC is determined as the first well without the visibility of turbidity in the broth for the fungicide (mimetic compounds), or the first well without the increase of OD at 600nm for fluconazole. A sample (25µL) from the well defined as the MIC, in addition with the wells with three higher concentrations, are plated onto YPD agar. Colonies were counted after 24 hours. The MFC (Minimal fungicidal concentration) is defined as the lowest concentration at which no colonies are observed. All MIC/MFC assay were performed in duplicate.

Fungicidal Kinetics

Fresh cultures of *Candida* were resuspended in RPMI-MOPS at a concentration of 2.5×10^6 CFU/ml. Samples (500µL) were incubated in the presence of the mimetic at 37°C, and aliquots were removed at the indicated timepoints, diluted in PBS and plated on YPD agar for colony counts after 24 hour growth at 37°C.

Resistance Assays

Fresh cultures of *Candida* (2.5×10^6 CFU/ml) were grown in 3ml RPMI-1640-MOPS in the presence of $0.5 \times$ MIC of each compound at 37°C shaking incubator for 24 hours. 50µl of the culture were plated on YPD agar and incubated at 37°C overnight. Colonies were resuspended in RPMI-MOPS and cultured under the same conditions in $0.5 \times$ MIC. This culturing procedure was repeated serially for 20 passages. MIC assays were performed at every other passage.

Analysis of *C. albicans* Biofilms

The denture biofilm model is carried out as described (Chandra, et al., 2001, Chandra *et al.*, 2009). Briefly, 8cm by 6cm double thickness strips of medium thickness pink dental base plate wax (Benco Dental, Wilkes-Barre, Penn. USA) were pressed onto a wet paper towel heated on a hot plate to 65°C to give the pressed side a slightly rough surface texture. The wax strips were packed, flaked and processed into denture base acrylic (Lucitone 199® Dentsply International, Milford, DE. USA). The resulting strips of denture acrylic had a smooth surface and a textured surface that resembled the tissue-facing aspect of a denture. These strips were then cut into 2cm squares using a low speed saw (Isomet®, Buehler Ltd., Lake Bluff, Ill. USA) and diamond blade with water lubricant. The cut surfaces were then polished using 600 grit silicon carbide paper (also from Buehler Ltd.) and water lubricant. The acrylic squares were stored in sterile water until use.

The denture squares were pre-coated with pooled (n=3), clarified human saliva for 30' at 37°C. Saliva was removed, and *C. albicans* were diluted to a concentration of 10^6 cfu/ml, and 100µl was placed on the squares. The squares were incubated at 37°C in a humid chamber for 3 hours. Non-adherent cells were removed, and the squares were further incubated in 6-well plates in 2.5ml RPMI-MOPS medium for 72 hours at 37°C, 100% humidity. For treatment, medium was replaced with fresh RPMI-MOPS containing the peptide mimetics. To quantify viability, an XTT assay was carried out using the TOX-2 kit (Sigma) diluted according to the manufacturer's instructions in RPMI without Phenol red, and incubated for 3 hours at 37°C. Metabolic activity was quantified by measuring optical density at 450nm and 600nm. Parallel cultures were scraped with a rubber scraper, resuspended in PBS, and plated on YPD-agar to quantify viable colonies.

To visualize activity microscopically, *Candida* are cultured in YPD agar plate to log phase and resuspended in RPMI-MOPS at the density of 10^8 cells/ml. Pooled, clarified human saliva was added to chamber slides (Labtek) for three hours. 200 μ l of the resuspended *Candida* was added to the chamber after the removal of saliva. Cells were incubated for 3 hours, and non-adherent cells were removed, and the slides were washed with RPMI-MOPS. The slides were cultured for a period of 72 hours before the treatment. The biofilms were visualized using the LIVE/DEAD *BacLight* Bacterial Viability kit (Molecular Probes) as described (Jin *et al.*, 2005). Stained slides were incubated at 30°C dark chamber for 20min and the pictures were captured using with a Zeiss LSM 510 on Zeiss Axiovert 100M Base.

Results

Fungicidal activity of mimetics against *C. albicans*

After assessing the MIC of 12 different compounds, the fungicidal kinetics of the four most active compounds were tested at 50 μ g/ml (figure 1A). Since no reduction in cfu was observed by 10 minutes with PMX3001016, only the three remaining compounds were also tested at 10 μ g/ml (B). Of the compounds tested, mPE exhibits the most rapid killing, with a three-log reduction in cfu after 1-5 minutes at 50 μ g/ml. When this compound was incubated with *C. albicans* hyphae, no viable cells were visible at concentrations higher than 32 μ g/ml after 24 hour incubation (data not shown), indicating that the compound could also kill cells in this form.

Activity against *C. albicans* biofilms

To examine the ability of the compounds to kill *C. albicans* in a biofilm, cells were grown on saliva-coated squares of polymethylmethacrylate as described (Chandra *et al.*, 2001). They were then treated with increasing concentrations of either mPE or PMX30016 for 24 hours, and cell viability was measured by XTT assay. The results in figure 2A clearly demonstrate that incubation with both compounds resulted in a significant reduction of viable *C. albicans* in this model of *Candida* infection. To confirm that the reduction in the metabolic activity measured by the XTT assay reflects *Candidacidal* activity, a parallel biofilm culture treated with PMX30016 was scraped from the denture material, dispersed by vortex and plated to determine viable blastoconidia. The results in figure 2B show a direct relationship between metabolic activity as measured by XTT assay and viable organisms.

To visualize the killing, we treated simple biofilms of *C. albicans* grown on saliva-coated plastic with 0, 50 or 100 μ g/ml mPE for 24 hours. The medium was removed, and the *Candida* were stained with SYTO9 and propidium iodide and visualized by fluorescence microscopy. While this stain is usually used to visualize bacterial viability, it has been demonstrated to be effective in studying *Candida* biofilms as well (Jin, et al., 2005). In this case, the green dye (SYTO9) stains cells regardless of viability, while the red dye (propidium iodide) stains only cells with compromised membranes. The results in Figure 2C demonstrate that mPE treatment results in increased membrane permeability, which allows the red fluorescent stain entrance into the fungi. Further examination of biofilms that were treated with 100 μ g/ml mPE for 0, 1 or 5 minutes showed the rapid development of membrane permeability, with visible staining within 1 minute of exposure (Figure 2D)

Development of resistance

To determine whether growth in sub-MIC concentrations of the drugs would lead to resistant organisms, we grew *C. albicans* 90028 in 0.5 $\times$ MIC of the compounds (or fluconazole as a positive control) for 20 serial passages. After every second passage, the MIC was quantified on the growing organisms. The results shown in figure 3 demonstrate that no resistance developed over the passages, in contrast to fluconazole, which rapidly led to resistance.

Factors that modify in vitro activity

Since a variety of physiological factors, including salt, serum proteins and factors found in saliva, are known to inhibit antimicrobial activity, we determined the effect of these factors on the activity of the compounds against *C. albicans*. Our results shown in table 1 demonstrate that there was no inhibition observed by saliva (and actually some increase in activity). These results support the further examination of the compounds as oral anti-fungal drugs. While other antimicrobial peptides exhibit reduced anti-candidal activity in the presence of salt (Vylkova *et al.*, 2007), our assays are carried out in physiological salt (154mM), demonstrating that there is no detrimental effect of salt on this activity.

To examine the potential for combination therapy with other antifungals, we determined the activity of mPE and PMX30016 in the presence of the common oral antiseptic agent, chlorhexidine and the standard antifungal agent, itraconazole. The results in table 2 demonstrate that the compound exhibits potent synergistic activity (i.e., an FIC index<1) with itraconazole, suggesting that it could be used in conjunction with this compound

Discussion

Oral candidiasis is routinely treated with topical and systemic antifungal agents including azoles such as fluconazole and itraconazole (Vazquez, 2000). However, resistance to these agents is a common occurrence, and together with the immunodeficiency often found in these patients, failure of standard therapies suggests novel strategies. Since their discovery, AMPs have been suggested as a useful source of antimicrobial therapeutic agents, especially due to the low development of resistance (Diamond, et al., 2009)). Histatins are naturally occurring antifungal peptides found in human saliva, that exhibit potent activity against *C. albicans* (Oppenheim *et al.*, 1988) (Helmerhorst *et al.*, 1997). Anti-candidal activity of other AMPs such as β -defensins (Vylkova *et al.*, 2007), cathelicidins (Benincasa *et al.*, 2006) and piscidins (Sung *et al.*, 2008) has also been described. Indeed, the normal expression of β -defensins may be necessary for natural defense against *Candida* growth (Conti *et al.*, 2009). However, numerous technical and biological issues have made their development into useful drugs difficult. We have previously demonstrated that small mimetics based on AMP structures are highly active against a wide variety of bacterial species, including biofilms of *Streptococcus mutans* (Beckloff *et al.*, 2007, Tew *et al.*, 2006). Here we show proof-of-principle for these compounds exhibiting potent antifungal activity against *Candida* species, in both planktonic and biofilm forms, and are active in the presence of saliva.

The rapidity with which killing is observed, together with the evidence that membrane permeability occurs with a short exposure suggests that these mimetics, like their AMP models, act on membranes, although this might not be the primary target of some peptides (for review see (Brogden, 2005)). Indeed, the phenylethynylene derivative we use here, mPE (Nusslein *et al.*, 2006, Arnt *et al.*, 2006), is able to cause leakage of dyes from phospholipid vesicles, at concentrations similar to those required to kill bacteria. It also promotes the loss of the membrane potential in bacteria at concentrations and over a time course consistent with its antimicrobial activity. Furthermore, the lack of resistance development after 20 passages at sub-MIC levels, similar to what is found with many peptides, is suggestive of physical action on membranes, as opposed to intracellular activity against a metabolic target such as is seen with histatins (Kavanagh and Dowd, 2004). This is supported by recent work using short antimicrobial peptides, demonstrating membrane activity as determined by dye-leakage and membrane depolarization experiments, as well as visualization by electron microscopy (Zhou *et al.*). Further studies on the mechanism of action of these mimetics, both against single organisms and on the biofilms, will allow a direct comparison with peptides, and will lead to a greater understanding of their activity in order to design the optimal structures.

Together, our results support the development of these compounds as therapies against oral infections such as candidiasis. The low toxicities against mammalian cells (Tew *et al.*, 2006), activity against biofilms grown on denture materials, and the lack of resistance found with the mimetics are important features for new anti-Candida agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. Isaac Rodriguez-Chavez, Director, NIDCR AIDS and Immunosuppression Program, for his scientific input in this manuscript. The authors thank Dr. David Perlin and Dr. Steven Park of the Public Health Research Institute for their helpful advice. This work was supported by US Public Health Service grant R43 DE18371 to RS and GD.

References

- Rodu B, Carpenter JT, Jones MR. The pathogenesis and clinical significance of cytologically detectable oral Candida in acute leukemia. *Cancer*. 1988; 62:2042–2046. [PubMed: 3167816]
- Dupont B, Graybill JR, Armstrong D, Laroche R, Touze JE, Wheat LJ. Fungal infections in AIDS patients. *J Med Vet Mycol*. 1992; 30 1:19–28. [PubMed: 1474444]
- Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. Candida-associated denture stomatitis. Aetiology and management: a review. Part 2. Oral diseases caused by Candida species. *Aust Dent J*. 1998; 43:160–166. [PubMed: 9707778]
- Edgerton M, Scannapieco FA, Reddy MS, Levine MJ. Human submandibular-sublingual saliva promotes adhesion of Candida albicans to polymethylmethacrylate. *Infect Immun*. 1993; 61:2644–2652. [PubMed: 8500903]
- Chandra J, Mukherjee PK, Leidich SD, Faddoul FF, Hoyer LL, Douglas LJ, Ghannoum MA. Antifungal resistance of candidal biofilms formed on denture acrylic in vitro. *J Dent Res*. 2001; 80:903–908. [PubMed: 11379893]
- Ghannoum MA, Rice LB. Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clin Microbiol Rev*. 1999; 12:501–517. [PubMed: 10515900]
- Diamond G, Beckloff N, Weinberg A, Kisich KO. The roles of antimicrobial peptides in innate host defense. *Curr Pharm Des*. 2009; 15:2377–2392. [PubMed: 19601838]
- Selsted ME, Szklarek D, Ganz T, Lehrer RI. Activity of rabbit leukocyte peptides against Candida albicans. *Infect Immun*. 1985; 49:202–206.
- Benincasa M, Scocchi M, Pacor S, Tossi A, Nobili D, Basaglia G, Buseti M, Gennaro R. Fungicidal activity of five cathelicidin peptides against clinically isolated yeasts. *J Antimicrob Chemother*. 2006; 58:950–959. [PubMed: 17023499]
- Zasloff M. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proc Natl Acad Sci U S A*. 1987; 84:5449–5453. [PubMed: 3299384]
- Oppenheim FG, Xu T, McMillian FM, Levitz SM, Diamond RD, Offner GD, Troxler RF. Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. *J Biol Chem*. 1988; 263:7472–7477. [PubMed: 3286634]
- Wakabayashi H, Abe S, Okutomi T, Tansho S, Kawase K, Yamaguchi H. Cooperative anti-Candida effects of lactoferrin or its peptides in combination with azole antifungal agents. *Microbiol Immunol*. 1996; 40:821–825. [PubMed: 8985937]
- Burrows LL, Stark M, Chan C, Glukhov E, Sinnadurai S, Deber CM. Activity of novel non-amphipathic cationic antimicrobial peptides against Candida species. *J Antimicrob Chemother*. 2006

- Nikawa H, Fukushima H, Makihira S, Hamada T, Samaranayake LP. Fungicidal effect of three new synthetic cationic peptides against *Candida albicans*. *Oral Dis*. 2004; 10:221–228. [PubMed: 15196144]
- Takakura N, Wakabayashi H, Ishibashi H, Teraguchi S, Tamura Y, Yamaguchi H, Abe S. Oral lactoferrin treatment of experimental oral candidiasis in mice. *Antimicrob Agents Chemother*. 2003; 47:2619–2623. [PubMed: 12878528]
- Meiller TF, Hube B, Schild L, Shirtliff ME, Scheper MA, Winkler R, Ton A, Jabra-Rizk MA. A novel immune evasion strategy of *Candida albicans*: proteolytic cleavage of a salivary antimicrobial peptide. *PLoS One*. 2009; 4:e5039. [PubMed: 19352427]
- Rotem S, Mor A. Antimicrobial peptide mimics for improved therapeutic properties. *Biochim Biophys Acta*. 2008
- Som A, Vemparala S, Ivanov I, Tew GN. Synthetic mimics of antimicrobial peptides. *Biopolymers*. 2008; 90:83–93. [PubMed: 18314892]
- Beckloff N, Laube D, Castro T, Furgang D, Park S, Perlin D, Clements D, Tang H, Scott RW, Tew GN, Diamond G. Activity of an Antimicrobial Peptide Mimetic against Planktonic and Biofilm Cultures of Oral Pathogens. *Antimicrob Agents Chemother*. 2007; 51:4125–4132. [PubMed: 17785509]
- Liu D, Choi S, Chen B, Doerksen RJ, Clements DJ, Winkler JD, Klein ML, DeGrado WF. Nontoxic membrane-active antimicrobial arylamide oligomers. *Angew Chem Int Ed Engl*. 2004; 43:1158–1162. [PubMed: 14983462]
- Tang H, Doerksen RJ, Tew GN. Synthesis of urea oligomers and their antibacterial activity. *Chem Commun (Camb)*. 2005:1537–1539. [PubMed: 15770251]
- Tang H, Doerksen RJ, Jones TV, Klein ML, Tew GN. Biomimetic facially amphiphilic antibacterial oligomers with conformationally stiff backbones. *Chem Biol*. 2006; 13:427–435. [PubMed: 16632255]
- Chandra J, Mukherjee PK, Ghannoum M. In Vitro growth and analysis of *Candida* biofilms. *Nat Protoc*. 2009; 3:1909–1924. [PubMed: 19180075]
- Jin Y, Zhang T, Samaranayake YH, Fang HH, Yip HK, Samaranayake LP. The use of new probes and stains for improved assessment of cell viability and extracellular polymeric substances in *Candida albicans* biofilms. *Mycopathologia*. 2005; 159:353–360. [PubMed: 15883718]
- Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol*. 2001; 183:5385–5394. [PubMed: 11514524]
- Vylkova S, Jang WS, Li W, Nayyar N, Edgerton M. Histatin 5 initiates osmotic stress response in *Candida albicans* via activation of the Hog1 mitogen-activated protein kinase pathway. *Eukaryot Cell*. 2007; 6:1876–1888. [PubMed: 17715369]
- Vazquez JA. Therapeutic options for the management of oropharyngeal and esophageal candidiasis in HIV/AIDS patients. *HIV Clin Trials*. 2000; 1:47–59. [PubMed: 11590489]
- Helmerhorst EJ, Van't Hof W, Veerman EC, Simoons-Smit I, Nieuw Amerongen AV. Synthetic histatin analogues with broad-spectrum antimicrobial activity. *Biochem J*. 1997; 326(Pt 1):39–45. [PubMed: 9337848]
- Vylkova S, Nayyar N, Li W, Edgerton M. Human beta-defensins kill *Candida albicans* in an energy-dependent and salt-sensitive manner without causing membrane disruption. *Antimicrob Agents Chemother*. 2007; 51:154–161. [PubMed: 17074797]
- Sung WS, Lee J, Lee DG. Fungicidal effect of piscidin on *Candida albicans*: pore formation in lipid vesicles and activity in fungal membranes. *Biol Pharm Bull*. 2008; 31:1906–1910. [PubMed: 18827353]
- Conti HR, Shen F, Nayyar N, Stocum E, Sun JN, Lindemann MJ, Ho AW, Hai JH, Yu JJ, Jung JW, Filler SG, Masso-Welch P, Edgerton M, Gaffen SL. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Exp Med*. 2009; 206:299–311. [PubMed: 19204111]
- Tew GN, Clements D, Tang H, Arnt L, Scott RW. Antimicrobial activity of an abiotic host defense peptide mimic. *Biochim Biophys Acta*. 2006; 1758:1387–1392. [PubMed: 16626628]

- Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol.* 2005; 3:238–250. [PubMed: 15703760]
- Nusslein K, Arnt L, Rennie J, Owens C, Tew GN. Broad-spectrum antibacterial activity by a novel abiogenic peptide mimic. *Microbiology.* 2006; 152:1913–1918. [PubMed: 16804167]
- Arnt L, Rennie J, Linser S, Wilumeit R, Tew GN. Membrane activity of biomimetic facially amphipathic antibiotics. *J Phys Chem B.* 2006; 110:3527–3532. [PubMed: 16494408]
- Kavanagh K, Dowd S. Histatins: antimicrobial peptides with therapeutic potential. *J Pharm Pharmacol.* 2004; 56:285–289. [PubMed: 15025852]
- Zhou C, Qi X, Li P, Chen WN, Mouad L, Chang MW, Leong SS, Chan-Park MB. High potency and broad-spectrum antimicrobial peptides synthesized via ring-opening polymerization of alpha-aminoacid-N-carboxyanhydrides. *Biomacromolecules.* 11:60–67. [PubMed: 19957992]

Fig. 1. Kinetics of activity against *C. albicans*

5×10^5 cfu *C. albicans* ATCC90028 were incubated with the indicated compounds at either 50 µg/ml (A) or 10 µg/ml (B) for the times indicated. Viable cfu were quantified by plating. Results shown are the mean fold reduction in cfu +/- standard deviation of three independent experiments.

Fig. 2. Activity of peptide mimetics against biofilms of *C. albicans*

Biofilms were grown on saliva-coated denture material (A, B) or in saliva-coated wells of a chamber slide (C, D) for three days. Compounds were added to the growth medium for 24

hours, and metabolic activity was measured by XTT assay (n=3; results are mean +/- standard deviation) (A). In panel B, activity was measured by incubating biofilms with PMX30016, followed by plating the fungi to quantify viable organisms (results shown are representative of three independent experiments). Visualization of killing was carried out by Live/Dead staining followed by confocal fluorescent microscopy (C). D, Time course of exposure to 100µg/ml mPE with Live/Dead staining.

Fig. 3. Development of resistance to mimetics

C. albicans 90028 was grown in 0.5× MIC of the compounds listed, at 35°C. At every second passage, a sample was tested for MIC by standard methods.

Table 1
IN VITRO ACTIVITY OF MIMETICS IN SALIVA

Saliva concentration (%)	mPE	Fold change in MIC		
		PMX30016	PMX519	PMX10149
0	0	0	0	0
10	0.5	0	0	0
25	0.5	0.5-0	1	0
50	0.5	0.5	1	0

Table 2
SYNERGISTIC ACTIVITY OF MIMETICS

Compound 1	Compound 2	FIC Index
mPE	Itraconazole	0.5
PMX30016	Itraconazole	0.2
PMX30016	Chlorhexidine	1
mPE	Chlorhexidine	1
mPE	PMX30016	1