Chemical Mimetics of Host Defense Proteins

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OTC: BB PYMX

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Technology Background Antimicrobial Program

- **Develop small non-peptidic, fully synthetic mimics of the Host Defense Proteins (HDPs) as systemic and topical antibiotics**
  - Novel approach for bactericidal activity
  - Clinical lead: PMX30063 in Phase 2 clinical study for ABSSSI

- **Most life forms produce these antimicrobial peptides or HDPs**
  - Microbes, insects, amphibians, plants, animals, humans
  - First line of defense against bacterial infection
  - Produced in skin, mucosal surfaces, neutrophils
    - Act locally
  - Directly toxic – target/disrupt bacterial cell membranes
    - Intracellular targets
    - Immune modulatory activities
The biological activities of host defense proteins depend on an amphiphilic helix.

Capture structural and biological properties of HDPs using fully synthetic, nonpeptidic scaffolds and sidechains.

Not peptidomimetics.
Advantages: Mimetic Approach

- **Narrow and broad-spectrum antimicrobial agents have been produced**
  - 0.5 to 2 µg/ml MICs vs Gram-positives
  - 0.5 to 8 µg/ml MICs vs Gram-negatives

- **Wide selectivity for bacteria over mammalian cells**
  - Significant improvements in cytotoxicity versus HDPs
  - >100 to 1,000 fold selectivities

- **Medicinal chemistry enables “fine-tuning” for specific activities**

- **Straightforward synthesis**
  - Common starting materials

- **Metabolically stable and active systemically in vivo**

- **Developed for systemic and topical uses**
Features of the PMX Compound Library

- **Fully-synthetic, nonpeptidic mimics of HDPs**
  - Capture structure/function on small nonpeptidic scaffold

- **Multiple compound series; defined by the backbone**
  - arylamides; arylureas, tricyclics, phenylalkynes, salicylamides

- **Restricted torsional freedom around axis critical**
  - Stabilize amphiphilic structure
  - Improves antimicrobial activity

- **Side chains provide high degrees of freedom for fine-tuning hydrophobicity and cationic balance**
<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R1</th>
<th>Modification</th>
<th>MIC (µg/ml)</th>
<th>Cytotoxicity (EC$_{50}$ µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC</td>
<td>SA</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>A</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>B</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>C</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>D</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>E</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

EC: *E. coli* 25922; SA: *S. aureus* 27660; EF: *E. faecalis* 29212; PA: *P. aeruginosa* 10145; KP: *K. pneumonia* 13883; 3T3: mouse 3T3 fibroblasts; HG2: human HepG2 liver cells; RBCs: isolated human erythrocytes
Mechanism of Action: Membrane Target

Membrane activity in Gram-positive and Gram-negative organisms supported by:
- Coarse grain molecular dynamic simulations
- Vesicle leakage assays
- Membrane permeabilization and potentiation assays
- Transcriptional profiling
- Transmission electron microscopy

TEM of *P. aeruginosa* on SMAP29 (3 hrs)

Cidal concs. of a PMX mimic cause visible signs of vesiculation (blebbing) at the E. coli membrane.

Similar morphological response reported for SMAP29 and *P. aeruginosa*.

Mensa et al. 2011 Antibacterial Mechanism of Action of Arylamide Foldamers; AAC In Press.
Preclinical Programs

Development of Antimicrobial Therapeutics for:

- Foodborne and Biowarfare-Pathogens
- Biofilm Infections
- Oral Candidiasis
- Malaria
## Susceptibility of Category A and B Biopathogens

### Library screen: Activity vs. *B. anthracis*, limited coverage over Gram-negative pathogens

### Chemical optimization: Improved Gram-negative coverage
Mice were infected with *B. anthracis* via inhalation of aerosolized Ames spores (50x LD$_{50}$) on Day 0. 24 hours after infection, mice were treated (IP) with PMX test agents (20 mpk) once daily for 14 days or the positive control ciprofloxacin (30 mpk) twice daily for 14 days.

Survival was recorded over the treatment period, and for 14 days following treatment.

PMX243, a close analog of PMX30063, dosed once daily was fully-protective at day 30 and activity was comparable to cipro (9/10 survivors)

PMX231 and PMX196 were also highly active with 8/10 and 7/10 mice, respectively, surviving at day 30.

Spore burdens in the surviving mice were well below threshold values for re-infection and comparable to cipro.
**Activity in vitro on 2 Day MRSA Colony Biofilm Cultures**

2 day biofilm cultures of MRSA 33591 established on filter discs

Discs are rinsed and incubated with compounds for 24 hours

Treated biofilms are rinsed, sonicated and viable cfus quantitated by plating

**MBEC: Minimal Biofilm Eradication Concentration (LOD = 400 cfus/ml)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC/MBC (µg/ml)</th>
<th>MBEC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMX231</td>
<td>1/2</td>
<td>32</td>
</tr>
<tr>
<td>PMX243</td>
<td>1/4</td>
<td>32</td>
</tr>
<tr>
<td>PMX519</td>
<td>1/1</td>
<td>32</td>
</tr>
<tr>
<td>rifampicin</td>
<td>&lt;0.01/0.1</td>
<td>&gt;128</td>
</tr>
<tr>
<td>tigecycline</td>
<td>1/&gt;16</td>
<td>&gt;128</td>
</tr>
<tr>
<td>daptomycin</td>
<td>1/1</td>
<td>128</td>
</tr>
<tr>
<td>gentamicin</td>
<td>1/2</td>
<td>64</td>
</tr>
</tbody>
</table>

Robust anti-biofilm activity was observed with the PMX mimics – including PMX231 and PMX243 - relative to standard antibiotics.
PMX-30063
Clinical Lead
PMX-30063: Activity Profile

*in vitro*

<table>
<thead>
<tr>
<th>PMX compound</th>
<th>MIC$_{90}$ (µg/ml)</th>
<th>Cytotoxicity (EC$_{50}$ µg/ml)</th>
</tr>
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<tbody>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBCs</td>
<td>3T3</td>
<td>HepG2</td>
</tr>
<tr>
<td>30063</td>
<td>1.0</td>
<td>&gt;500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>430</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,031</td>
</tr>
</tbody>
</table>

**Low cytotoxicity**

Rapid bactericidal activity; 0.5 to 4 hrs.

Low risk for resistance development
PMX-30063 vs *Staph.* sp. with Defined Susceptibility Phenotypes

*Broad spectrum activity against drug-sensitive and resistant strains of S. aureus, S. epidermidis and S. hemolyticus*

<table>
<thead>
<tr>
<th></th>
<th>Drug-suscept.</th>
<th>OXA-R</th>
<th>VRSA/VISA OXA-R</th>
<th>LZD-NS OXA-R</th>
<th>DAP-NS OXA-R</th>
<th>VRSA/VISA DAP-NS OXA-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>isolates</td>
<td>217</td>
<td>161</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>30063</td>
<td>0.25 - 1</td>
<td>0.25 - 2</td>
<td>0.5 - 1</td>
<td>0.5 - 1</td>
<td>0.5 - 2</td>
<td>0.5 - 1</td>
</tr>
</tbody>
</table>

OXA-R: oxacillin-resistant  
VISA: vancomycin intermediate S. aureus  
VRSA: vancomycin resistant S. aureus  
LZD-NS: linezolid non-susceptible  
DAP-NS: daptomycin non-susceptible
PMX-30063: Pharmacological Properties

- Rapid bactericidal activity, 0.5 – 4 hr. time-kills
- Low resistance potential
- Well tolerated in vivo
- Suitable and predictable PK properties (Poster # A2-035; Saturday 11:30 AM)
- Long post-antibiotic effect in vivo
- Metabolically stable

PK/PD parameters derived from animals models: AUC and C\textsubscript{max} drivers for efficacy

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Amt. of drug needed to achieve maximum-efficacy in animal-models (AUC\textsubscript{free} hr*ug/ml)</th>
<th>Human equivalent drug exposure (AUC\textsubscript{free} hr*ug/ml)</th>
<th>Human single dose (mg/kg)</th>
<th>Phase 1 Total Maximum Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA\textsuperscript{1}</td>
<td>0.5 – 1.3</td>
<td>1.6</td>
<td>0.15</td>
<td>2.5 (1 day)</td>
</tr>
<tr>
<td>MSSA\textsuperscript{2}</td>
<td>1.9</td>
<td>2.2</td>
<td>0.20</td>
<td>3.0 (over 5-10 days)</td>
</tr>
<tr>
<td>MRSA\textsuperscript{1}</td>
<td>1.7 – 3.0</td>
<td>2.8</td>
<td>0.25</td>
<td>5.55 (over 14 days)</td>
</tr>
</tbody>
</table>

\textsuperscript{1} mouse thigh burden; \textsuperscript{2} rat granuloma pouch
PMX-30063: Clinical Development

- **Three phase 1 studies completed**
  - Ascending single (PMX63-101) and multiple doses for 5 days (PMX63-102) and fixed dose for 14 days (PMX63-103, poster A2-035)
  - IV infusions 48H, 24H and 12H

- **Pharmacokinetics**
  - Consistent and linear pharmacokinetics in plasma with half-live of ~ 23 hours. No apparent gender difference in drug disposition. Renal excretion does not appear to be a significant elimination pathway.

- **Ex-vivo efficacy**
  - Long lasting bactericidal and inhibitory activity against MSSA and MRSA detected in serum from human subjects following administration of doses as low as 0.1 mg/kg

- **Safety**
  - Paraesthesia and hypoaesthesia (numbness and tingling) with acute onset and rapid resolution; No neurotoxicity evident in human subjects or animal safety studies.
  - Increase in blood pressure and heart rate reversible after treatment discontinuation

- **Phase 2 in ABSSSI ongoing**
  - www.clinicaltrials.gov: NCT01211470
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