

PolyMedix, Inc.

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SUMMARY

- A series of arylamide compounds with MWs < 1,000 D. that mimic the structural properties of antimicrobial peptides have potent antibacterial activities.
- Gram-positive organisms appear the most susceptible but Gram-negative activity is found with several of the discovery lead compounds. Activity against anaerobic organisms (*C. difficile* and *P. acnes*) is also present.
- The compounds are bactericidal and time kills range from 30 minutes to 4 – 6 hours, depending on the organism. Antimicrobial activities are largely independent of salt effects over physiological concentrations.
- The discovery lead compounds are selective for bacteria over mammalian cells, having selectivity ratios (EC_{50}/MIC) that range from 250 to >20,000.
- There was no evidence for the development of resistance in serial passage microbroth assays with *S. aureus*, MRSA or *P. aeruginosa*.
- Two discovery lead compounds that share common sidegroups but have different backbones (PMX10066 and PMX30006) are highly stable in the presence of human and mouse microsomal proteins.
- In single dose acute toxicity trials (MTD), the compounds are generally tolerated in the mouse and rat up to doses of 15 to 25 mg/kg. Infusion of PMX10129 for 1 or 4 hours significantly raises the MTD indicating that the associated adverse events are C_{max} -related.

- Several of the discovery lead compounds (PMX30006, PMX30016 and PMX10129) are highly efficacious in the mouse thigh burden model, achieving $\geq 4\log_{10}$ reductions in cfus at total doses of 6 mg/kg for PMX30006, 4 mg/kg for PMX10129 and 2 mg/kg for PMX30016.
- PMX30016 is highly efficacious in the mouse protection assay, fully protecting mice over a 7 day period following peritoneal infection at a total dose of 20 mg/kg.
- Pharmacokinetic analyses of PMX30006 administered by iv infusion in the rat demonstrate that blood levels of PMX30006 significantly above its MIC can be readily obtained for extended periods of time without overt toxicity, and its PK properties are suitable for an *iv* antibacterial therapeutic.

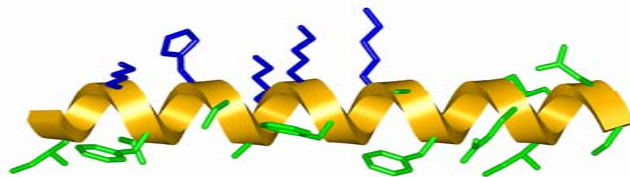
CONCLUSION

Fully synthetic compounds that mimic the structure of antimicrobial peptides have been designed and synthesized. Discovery lead compounds are active against a spectrum of Gram-positive organisms including clinical isolates of *S. aureus*, coagulase-negative *Staphylococcal* species, MRSA, *E. faecalis*, *E. faecium*, VRE strains, *C. difficile* and *P. acnes*. Activity against Gram-negative organisms is also present in the series but coverage is more limited. The compounds are bactericidal in action and rapidly kill both Gram-positive and Gram-negative organisms. Serial passage studies with several organisms have not demonstrated the appearance of emerging resistance. Discovery lead compounds are highly efficacious in a mouse thigh burden model when administered by iv bolus injection, and *in vivo* activity has also been demonstrated in a mouse protection assay following peritoneal infection and iv drug administration. The most *in vivo* potent compounds are highly efficacious at doses where no adverse signs of toxicity are evident, either in acute single dose toxicity studies (MTD) or in repeat dose 7 day toxicity studies where serum chemistry, hematology, blood coagulation, histopathological and behavioral endpoints were measured (data not shown). Additional repeat dose toxicity trials are in progress with

these and other discovery lead compounds to identify the preferred compound and dosing route (iv bolus vs iv infusion) that provides the optimal therapeutic index. These results support further development of these and other compounds in the series for use as iv antimicrobial agents.

INTRODUCTION

Antimicrobial peptides represent the first line of defense against microbes for many species. Although the peptides are composed of many different sequences, their physiochemical properties are remarkably similar. They adopt amphiphilic architecture with positively charged groups segregated to one side of the secondary structure and hydrophobic groups on the opposite surface. In fact, it is the overall physiochemical properties that are responsible for biological activity of these peptides and not the precise sequence



Hydrophobic residues are green (light), basic residues are blue (dark).

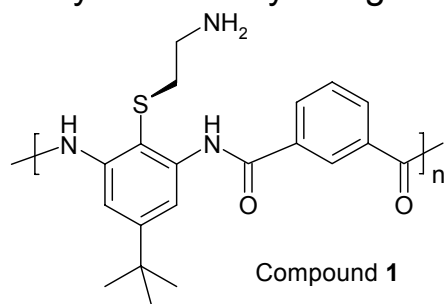
Schematic representation of the structure for the cationic and amphiphilic α -helical host defense peptide, magainin 1.

The antimicrobial activities of the host defense proteins have been linked to direct cytotoxic actions and modulation of innate immune system. Their direct antimicrobial activities are proposed to involve both membrane and non-membrane effects. Antimicrobial peptides have remained an effective weapon against bacterial infection over evolutionary time indicating that their mechanism of action thwarts bacterial responses which lead to resistance against toxic substances. This premise is supported by direct experimental data showing that no appreciable resistance to the action of the antimicrobial peptides occurs after multiple serial passages of bacteria in the presence of sub-lethal concentrations of the peptides.

Given their very broad specificity, amphiphilic antimicrobial peptides 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. Therefore, we have recently developed 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.

The goal of our synthetic approach is to capture the structural and biological properties of antimicrobial peptides within the framework of easily synthesized polymers and oligomers. In general, *de novo* design of oligomers was done by defining a three-dimensional framework of the backbone assembled from a repeating sequence of monomers using molecular dynamics and quantum force field calculations. Next, side groups were computationally grafted onto the backbone to maximize diversity and maintain drug-like properties. The best combinations of functional groups were then computationally selected to produce a cationic, amphiphilic structures. Representative compounds were synthesized from this selected library to verify structures and test their biological activity.

Many of the early design and testing studies focused on compound **1**.



This structure is amphiphilic by virtue of the hydrophobic t-butyl group (projecting from the bottom of the repeat unit) and a charged amino group (at the top of the repeat unit). Using this very simple framework, multiple series of oligomers were synthesized and found to be potently antimicrobial and selectively cytotoxic for bacterial versus mammalian cells. Subsequent chemical analoging efforts improved the biological activities of several of the series and results with these second and third generation compounds are described here.

6. Effects of Ionic Strength on Antimicrobial Activities

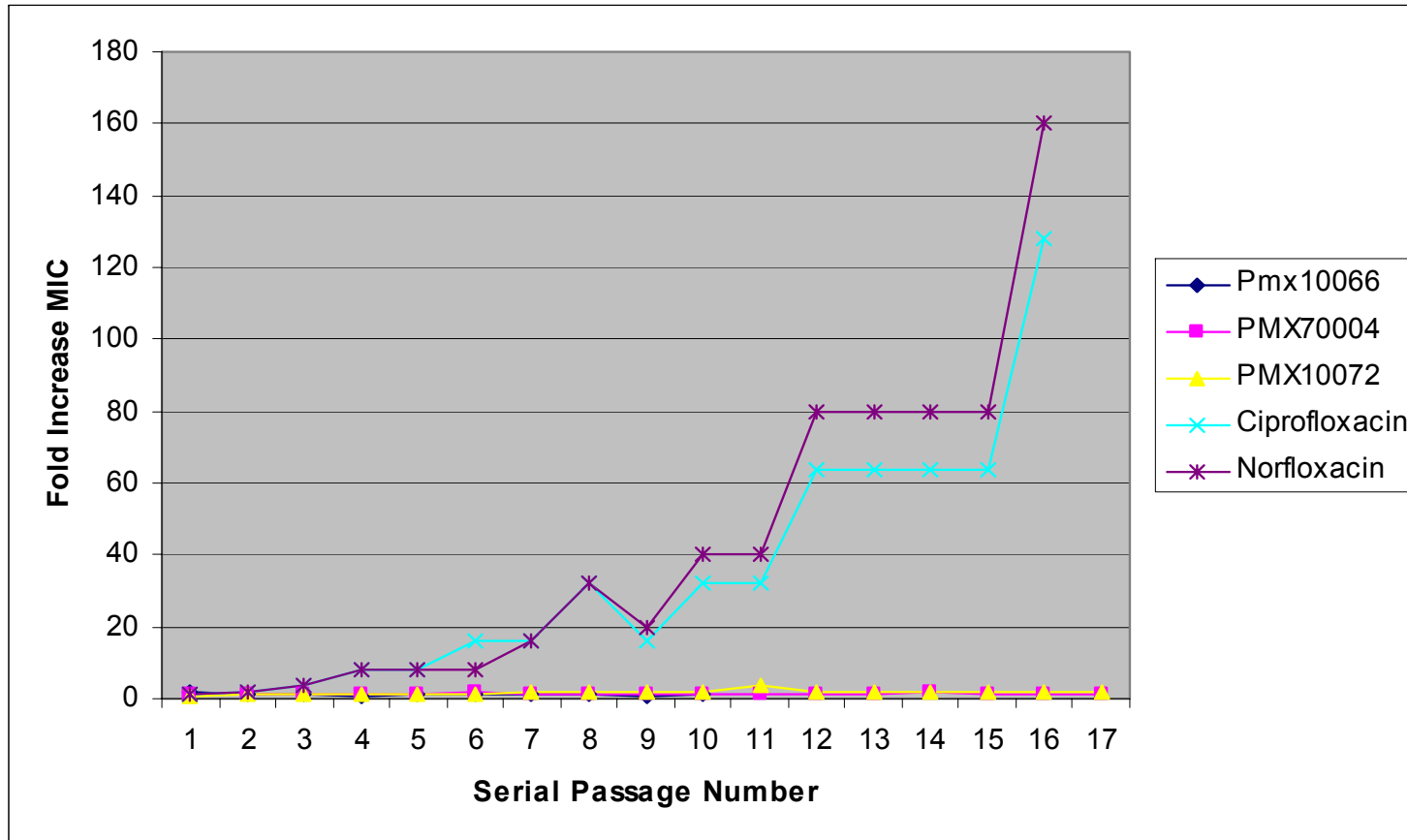
PMX Cmpd	No Ca ²⁺ or Mg ²⁺				20-25 ug/ml Ca ²⁺ /10-12.5 ug/ml Mg ²⁺			
	0 mM NaCl	10 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	10 mM NaCl	50 mM NaCl	150 mM NaCl
30006	0.049	0.049	0.049	0.098	0.049	0.049	0.049	0.098
30012	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
10066	0.195	0.098	0.195	0.39	0.195	0.195	0.195	0.39

Methods: MICs were determined in standard microbroth dilution assays according to CLSI guidelines with suggested modifications for testing cationic agents. Assays performed in the absence of divalent cations (Mg²⁺ and Ca²⁺) were done in Mueller Hinton I broth supplemented with NaCl to the indicated concentrations and assays performed in the presence of divalent cations were done in Mueller Hinton II broth supplemented with NaCl to the indicated concentrations.

Results: Many of the antimicrobial peptides, including members of the human cathelicidins and defensins, lose substantial antimicrobial activity in the presence of physiological salt concentrations and are only active under low salt conditions. Therefore, salt effects on the antimicrobial activities of the PMX compounds were tested. Three indicator compounds were selected; PMX10066 a benzene (all carbon) center ring analog with 4 guanidino end groups, PMX30006 a pyrimidine (nitrogen-containing) center ring analog with 4 guanidino end groups and PMX30012, a pyrimidine center ring analog with 2 guanidino end groups. In MIC experiments with varying levels of divalent (Ca²⁺/Mg²⁺) or monovalent (Na⁺) cations, antimicrobial activity is

comparable for all three compounds under low salt or high salt conditions. These results indicate that physiological ionic strengths should not adversely affect antibacterial activities of the PMX compounds.

7A: Development of Resistance in Serial Passage Assays: *S. aureus* (ATCC 29213)



Methods: Serial passage of all strains was performed over 17 successive overnight passages. Serial passage and MICs were performed in microtiter panels containing antimicrobials, each over a range of doubling dilution concentrations. Cation-adjusted Mueller-Hinton Broth was the broth used to inoculate panels. After the incubation period, the entire

content of the well (100 µl) with the highest concentration of compound (20 hours incubation) permitting visible growth was taken and diluted to the correct inoculum (5×10^5 CFU/mL), and a fresh panel re-inoculated with the appropriate inoculum.

7B: Development of Resistance in Serial Passage Assays: PMX30006 vs. MRSA and *P. aeruginosa*

PMX compound	Δ MIC (passage 1 to passage 17)	
	MRSA 1087076	<i>P. aeruginosa</i> ATCC 27853
10066	1 doubling dilution	1 doubling dilution
30006	1 doubling dilution	1 doubling dilution

Methods: Serial passage of both strains was performed over 17 successive overnight passages. Serial passage and MICs were performed in microtiter panels containing antimicrobials, each over a range of doubling dilution concentrations. Cation-adjusted Mueller-Hinton Broth was the broth used to inoculate panels. After the incubation period, the entire content of the well (100 µl) with the highest concentration of compound (20 hours incubation) permitting visible growth was taken and each subcultured in 5 ml of drug-free Cation-adjusted Mueller-Hinton Broth, allowed to grow until the turbidity of a 0.5 McFarland standard was approximated (2-6 hours) diluted to the correct inoculum (5×10^5 CFU/mL) according to the CLSI method (M7-A6), and a fresh panel re-inoculated with the appropriate inoculum. Alternatively the entire content of the well (100 µl) with the highest concentration of compound (20 hours incubation) permitting visible growth was plated on drug-free agar medium. After overnight incubation, the inoculum (5×10^5 CFU/mL) was prepared according to the CLSI method (M7-A6), and a fresh panel re-inoculated with the appropriate inoculum. This inoculum

approach was used in order to simplify the timing challenge that occurred because of the varying growth rates/times that were required for the drug-exposed organisms to reach a 0.5 McFarland. Experiments in (B) performed by Focus-Biolnova

Results: To investigate the potential for bacteria to develop resistance against the antimicrobial activity of the PMX compounds, *S. aureus* was serially passaged in 0.5x MIC concentrations of PMX10066 and a structurally-related compound, PMX10072, for up to 17 passages (A). An amphiphilic broad spectrum phenylalkyne compound, PMX70004, was also included in the assay. MIC values were determined at each passage. As a control, parallel cultures were also exposed to 0.5x MIC concentrations of ciprofloxacin or norfloxacin, two broad spectrum fluoroquinolones for which resistance has been reported in this experimental format. There is no change in the MICs for PMX70004, PMX10072 or PMX10066 over the entire 17 passage time course. Conversely, an increase in the MIC is readily observed by passage 4 for both ciprofloxacin and norfloxacin that reaches 128 and 160-fold, respectively, by passage 16 at which time the samples were terminated. PMX30006 has also been tested in a similar format with 2 additional organisms, Gram-positive MRSA and Gram-negative *P. aeruginosa* (B). Again, there was no significant increase in the MIC (1 doubling dilution) versus either organism. These results with the PMX compounds are very similar to results from resistance studies with the antimicrobial peptides and together demonstrate the difficulty bacteria have in building resistance to this mechanism of cell killing.

RESULTS

2. Antimicrobial and Cytotoxic Activities of Discovery Lead Compounds

Cmpd	MIC (ug/ml)						EC ₅₀ (ug/ml)		HC ₅₀
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. aureus</i> + serum	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	3T3	HepG2	RBCs
10066	6.25	0.4	0.8						468
30006	0.39	0.049	1.56	0.049	3.13	0.78	12, 31	28	25
30012	1.56	0.049	0.78	0.195	3.13	1.56	70	48	N.A.
30016	0.39	0.049	1.56	0.195	1.56	1.56	79, 147	341	56
10129	1.56	0.098	3.13	0.39	12.5	3.13	>2000	>2000	26
Melitinnin	N. D.	2	N. D.	N. D.	N. D.	N. D.	4	1	2

N. D.: Not Determined

Methods: MICs were determined in standard microbroth dilution assays according to CLSI guidelines with suggested modifications for testing cationic agents (Yan and Hancock. 2001. *Antimicrob. Agents Chemother.* 45: 1558-1560). The modifications were made to minimize loss of the antimicrobial agent due to adsorption onto glass or plastic surfaces and by precipitation at high concentrations. Mammalian cell cytotoxicities were tested in mouse 3T3 and human HepG2 cells in the absence of serum using an MTS viability assay that measures dehydrogenase enzymatic activity in metabolically active cells and defines EC₅₀ values representing compound concentrations that cause 50% lethality. EC₅₀ values are corroborated in trypan blue exclusion assays. Hemolysis (HC₅₀) was measured following incubation of human red blood cells in the presence of compound for 1 hour. Melitinnin, a lytic antimicrobial peptide, was used as a positive control agent. [*S. aureus* + serum] represents MICs done in the presence of 40% mouse serum.

Results: All newly synthesized compounds are tested in this primary screen of Gram-positive and Gram-negative organisms. The Gram-positive organisms (*S. aureus* and *E. faecalis*) appear to be most susceptible but Gram-negative activity is found with several of the discovery lead compounds (PMX30006, PMX30012 and PMX30016). Inhibition of antimicrobial activity ranging from 1 to 5 doubling dilutions is evident with inclusion of 40% serum. Subsequent animal studies (see below) have shown that MICs below 4 in the presence of 40% serum are necessary but not sufficient for antimicrobial efficacy *in vivo*. Preliminary ultrafiltration studies in serum and plasma demonstrate approximately 90% protein binding indicating a likely cause for the shift in MICs. PMX compounds are stable when incubated in the presence and absence of 100% mouse serum for 2 hours at 37°C.

Comparison of the EC₅₀ and HC₅₀ values with the MICs for *S. aureus* indicate high selectivity for the susceptible bacterial strains over mammalian cells (see below). The EC₅₀/MIC or HC₅₀/MIC ratios range from 250 to >20,000 and are useful for prioritizing compounds for further study.

PMX Compound	MIC (ug/ml)	Cytotoxicity (EC ₅₀ ug/ml)			Selectivity (EC ₅₀ /MIC)		
	<i>S. aureus</i>	RBCs	3T3 cells	HepG2	RBCs	3T3	HepG2
10066	0.4	468	102	146	1200	255	365
30006	0.049	25	21 (avg)	28	510	428	571
30012	0.049	N.D.	70	48	-	1428	980
30016	0.049	56	113 (avg)	341	1142	2306	6959
10129	0.098	26	>2000	>2000	>260	>20,000	>20,000
Melittin	2*	2	4	1	1	2	0.5

3. Clinical Isolate Screen: 2nd and 3rd Generation Compounds

Organism	MIC range (ug/ml)*	
	PMX30006	PMX10066
Methicillin-R <i>Staphylococcus aureus</i> (MRSA)	<0.12	0.5 - 1
<i>Staphylococcus epidermidis</i>	<0.12	0.25 - 1
<i>Staphylococcus haemolyticus</i>	<0.12	0.25
<i>Staphylococcus hominis</i>	<0.12	<0.12 – 0.5
<i>Staphylococcus saprophyticus</i>	<0.12	<0.12 – 0.25
<i>Streptococcus pneumoniae</i>	1 - 2	8 - 16
Penicillin-R (PEN-R) <i>Streptococcus pneumoniae</i>	2 - 8	16 - 32
<i>Streptococcus pyogenes</i>	0.5	0.5
<i>Enterococcus faecalis</i>	0.25 - 1	16 - 32
Vancomycin-R <i>E. faecalis</i> (VRE)	0.25 - 1	4 - 32
<i>Enterococcus faecium</i>	0.12 – 0.25	0.5 - 2
Vancomycin-R <i>E. faecium</i> (VRE)	0.25	2 - 8
Multidrug-R (MDR) <i>Pseudomonas aeruginosa</i>	1 - 8	8 - >32

* 3 isolates tested for each organism

Methods: MICs were determined in standard microbroth dilution assays according to CLSI guidelines with suggested modifications for testing cationic agents. Assays performed by Focus-Biolnova.

Results: PMX10066 is a second generation compound having a benzene center ring backbone. It is highly active against many of the Gram-positive organisms including the drug-resistant and coagulase-negative strains of *S. aureus*. PMX30006 is a third generation compound in which

rigidity along the backbone axis is increased by intramolecular hydrogen bonding between the nitrogens of the pyrimidine center ring and the hydrogens from the neighboring amide bonds. PMX30006 demonstrates even greater potency against the *S. aureus* strains and has a significantly broader spectrum of activity where potent antibacterial activity is now seen against the Gram-positive strains of *Enterococcus faecalis*, and the Gram-negative, multi-drug resistant strains of *Pseudomonas aeruginosa*.

4. Antimicrobial activity of PMX30006 against anaerobic clinical isolates

Organism	PMX30006	Clindamycin	
	MIC range (ug/ml)*	MIC range (ug/ml)*	CLSI
<i>Bacteroides fragalis</i>	8-16	0.25 - 2	S
<i>Clostridium difficile</i>	0.5	0.25 – 0.5	S
<i>Clostridium perfringens</i>	4	0.12 - 2	S
<i>Propionibacterium acnes</i>	0.12	0.06 – 0.12	S

* 3 isolates per organism.

Methods: MICs were determined in standard microbroth dilution assays according to CLSI guidelines for Brucella. Testing was done in Brucella broth supplemented with vitamin K, hemin and blood. Assays performed by Focus-Biolnova.

Results: The most susceptible anaerobic organisms for PMX30006 are the Gram-positive *C. difficile* and *P. acnes* isolates with MICs comparable to the positive comparator agent, Clindamycin. Testing with PMX30016 and PMX10129 is underway.

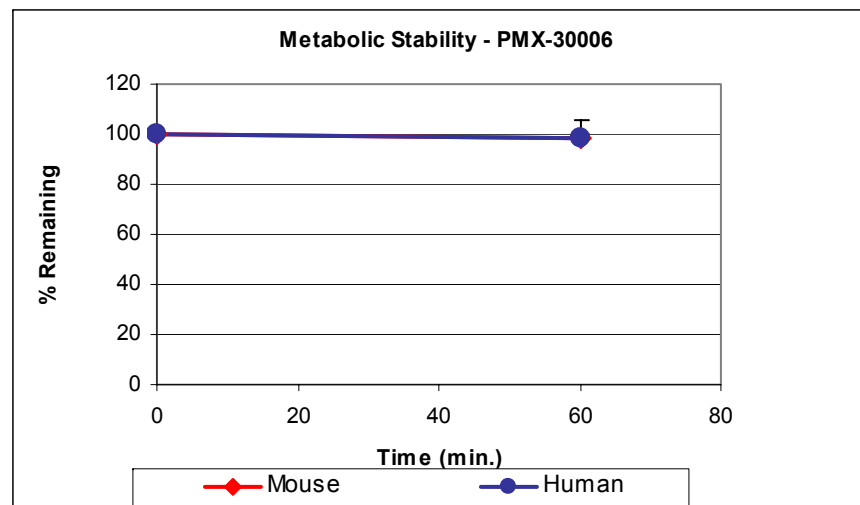
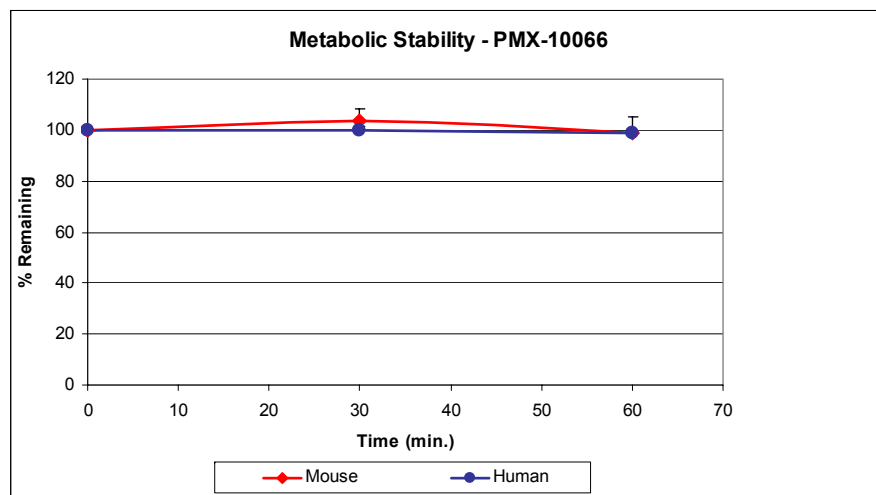
5. Time Kill Results with PMX Discovery Lead Compounds

Compound	<i>S. aureus</i>	<i>E. coli</i>	<i>B. cereus</i>
PMX30016	4 – 6 hrs	< 0.5 hrs	0.75 – 1.5 hrs
PMX10129	4 – 6 hrs	1 – 2 hrs	0.75 – 1.5 hrs

Methods: Values represent the time required for $> 3\log_{10}$ reductions in viable bacteria following addition of compound at 10x MIC. Bacteria were exposed to compound in broth cultures (x cfu/ml) and viable bacteria counts were determined in plating assays.

Results: The minimal bactericidal concentrations (MBC) for the discovery lead compounds have been measured in plating experiments following MIC determinations. In all cases tested, the MBC was either at the MIC or within one serial 2-fold dilution demonstrating bactericidal activity. Therefore, time-kill studies were done to further assess bactericidal actions. Gram-negative *E. coli* and Gram-positive *B. cereus* (MIC = x) are rapidly killed by the PMX compounds with time kills ranging from < 30 minutes to 2 hours. More moderate time-kills of 4 – 6 hours are observed with *S. aureus*.

8. Metabolic Stability in Isolated Human and Mouse Liver Microsomes



Methods: PMX10066 and PMX30006 were incubated with 0.5 mg/ml microsomal protein at 37°C with and without NADPH for 0, 30 and 60 minutes. Quantitations of parent compound were done using lc/msms methods developed for each compound. Experiments were performed at Ricerca Biosciences.

Results: The stabilities of PMX10066 and PMX30006 have been tested in the presence of human and mouse liver microsomal proteins. Little to no metabolism of either compound was observed in human and mouse microsomal preparations over the experimental time course. Although each lead molecule must be evaluated, these results indicate that compounds bearing benzene and pyrimidine center ring backbones are stable and liver metabolism should not be a confounding factor upon *in vivo* administration.

9. Maximum Tolerated Dose (MTD)

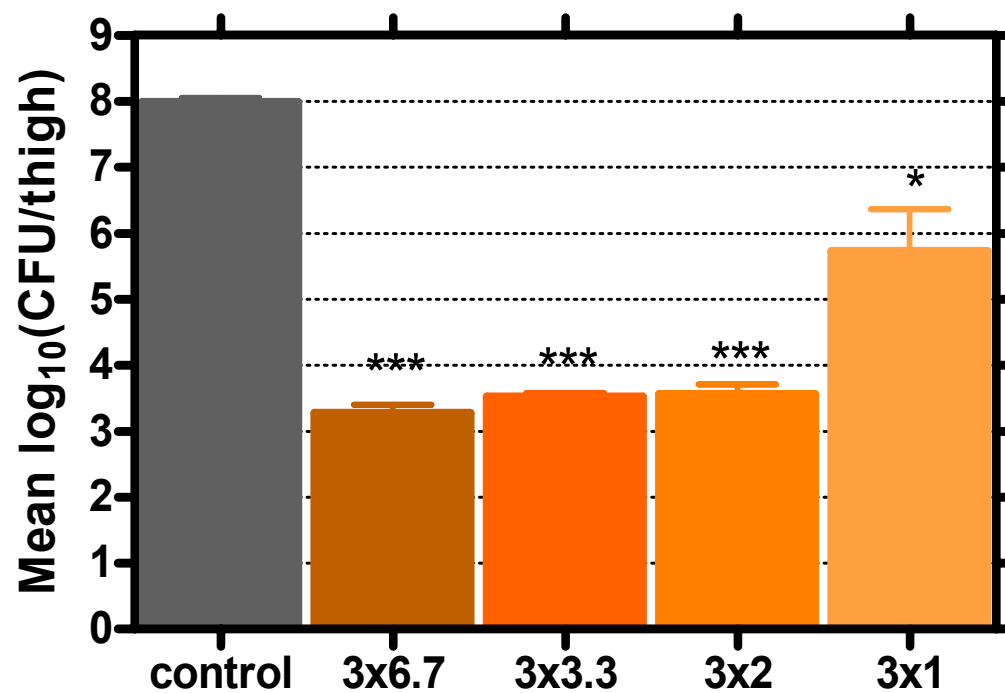
Dosage	MTD (mg/kg)	
	mouse	rat
PMX30006		
iv bolus	25	20
PMX30016		
iv bolus	20	N. D.
PMX10129		
iv bolus	15	10
iv infusion – 1 hr	N. D.	20 - 30
iv infusion – 4 hr	N. D.	45

Methods: For iv administrations, PMX30006 and PMX30016 were formulated in 1x phosphate buffered saline (PBS) and PMX10129 was formulated in 0.5x PBS. For iv bolus administration, compounds were injected in the tail vein at a volume of 2 ml/kg. For iv infusions in rats, compound was administered via a femoral vein catheter attached by a tether to the drug reservoir at a volume of 3 ml/kg. Acute toxicity trials with PMX30006 and PMX30016 were performed at Ricerca Biosciences. Acute toxicity trials with PMX10129 were performed at Covance Laboratories.

Results: Initially, limit dose determinations were made following *iv* bolus injection of compound in an ascending/descending dose study. At each dose, two to three mice or rats were administered compound in the tail vein and survival was monitored over two days. At toxic doses, apparent toxicity consistent with rapid hypotension was observed within 5 minutes of compound administration. Once the limit dose was determined, a single dose toxicity study was conducted to define the maximum tolerated dose (MTD) where clinical signs are recorded over a 4 to 7 day period following compound administration and gross necropsy is performed at the conclusion of the study. In all cases examined, no apparent toxicity was observed at the limit doses and the maximum tolerated dose was found to be the same as the limit dose. The sequentially higher MTDs observed with PMX10129 when administered by *iv* bolus, 1 hour and 4 hour infusions indicate that the MTD-related adverse events are primarily C_{max}-dependent.

10. Potent Activity of PMX Discovery Lead Compounds in the Mouse Thigh Burden Model

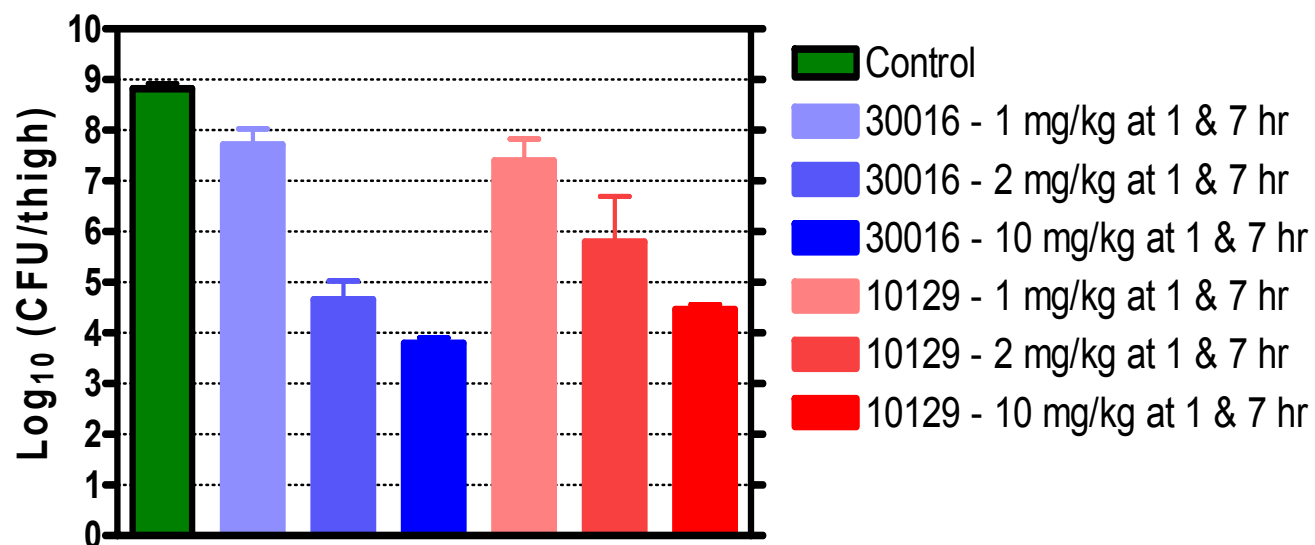
PMX30006



Neutropenic mice were inoculated in the posterior thigh muscles with *S. aureus* ATCC13709 at 1×10^6 cfu/thigh and then treated with PMX30006 by iv bolus injection in the tail vein at 1, 5 and 9 hours post infection (n = 4/group). Four total dose levels were evaluated; 20 mg/kg (6.7 mg/kg/dose), 10 mg/kg (3.3 mg/kg/dose), 6 mg/kg (2 mg/kg/dose) and 3 mg/kg (1 mg/kg/dose). PMX30006 was formulated in PBS and administered in a dose volume of 2 ml/kg. Bacterial quantitation was done 25 hours post infection by aseptically removing the thigh muscles, homogenizing and plating serial dilutions of the homogenate. Experiments were performed at Ricerca Biosciences.

Results: The mouse thigh burden model is a widely used animal model for evaluating antibacterial activity of preclinical compounds. When administered in a multiple (3) dose format at 6.7 mg/kg per dose, PMX30006 caused a significant reduction in bacterial cell count ($> 4 \log_{10}$) at 25 hours after infection ($P < 0.0001$). Furthermore, full efficacy with PMX30006 could be achieved with 3 doses at 2 mg/kg per dose ($P < 0.0001$) and significant efficacy was readily apparent with 3 doses of only 1 mg/kg per dose ($P = 0.0127$).

PMX30016 and PMX10129

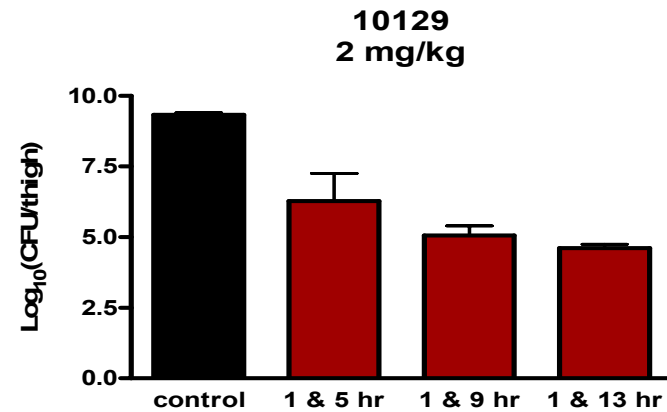
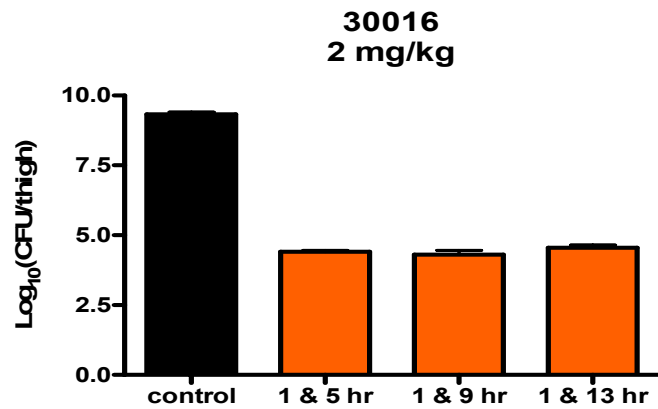
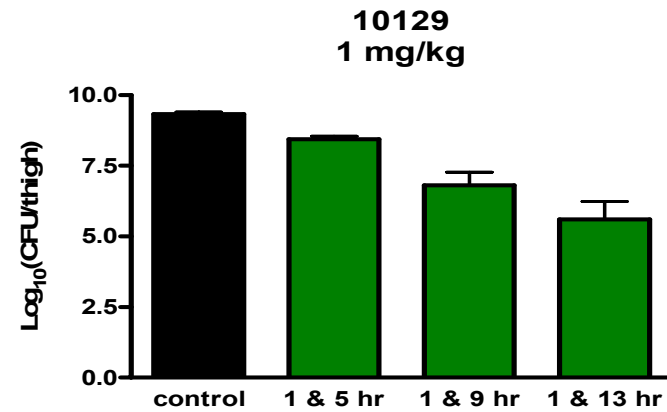
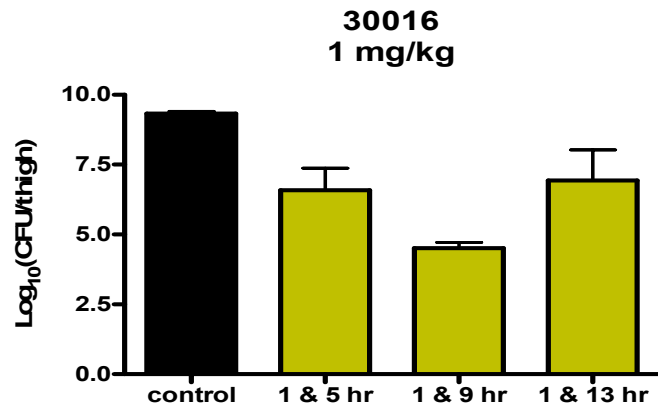


Methods: Neutropenic mice were inoculated in the posterior thigh muscles with *S. aureus* ATCC13709 at 1×10^6 cfu/thigh and then treated with PMX30016 or PMX10129 by iv bolus injection in the tail vein at 1 and 7 hours post infection (n = 4/group). Three total dose levels were evaluated; 20 mg/kg (10 mg/kg/dose), 4 mg/kg (2 mg/kg/dose), and 1 mg/kg (0.5 mg/kg/dose). PMX30016 was formulated in PBS and PMX10129 was formulated in 0.5x PBS. Both compounds were administered in a dose volume of 2 ml/kg. Bacterial quantitations were done 25 hours post infection. Experiments were performed at Ricerca Biosciences.

Results: Robust efficacy is observed with both PMX30016 and PMX10129. Efficacies for PMX30016 exceed $5\log_{10}$ and $4\log_{10}$ reductions in viable bacterial cell counts (colony forming units, cfu) with total

doses of 10 mg/kg and 4 mg/kg, respectively. PMX10129 is slightly less efficacious in this dosing format with reductions reaching 4.2 log₁₀ and 2.8 log₁₀ cfus at 10 mg/kg and 4 mg/kg total doses, respectively.

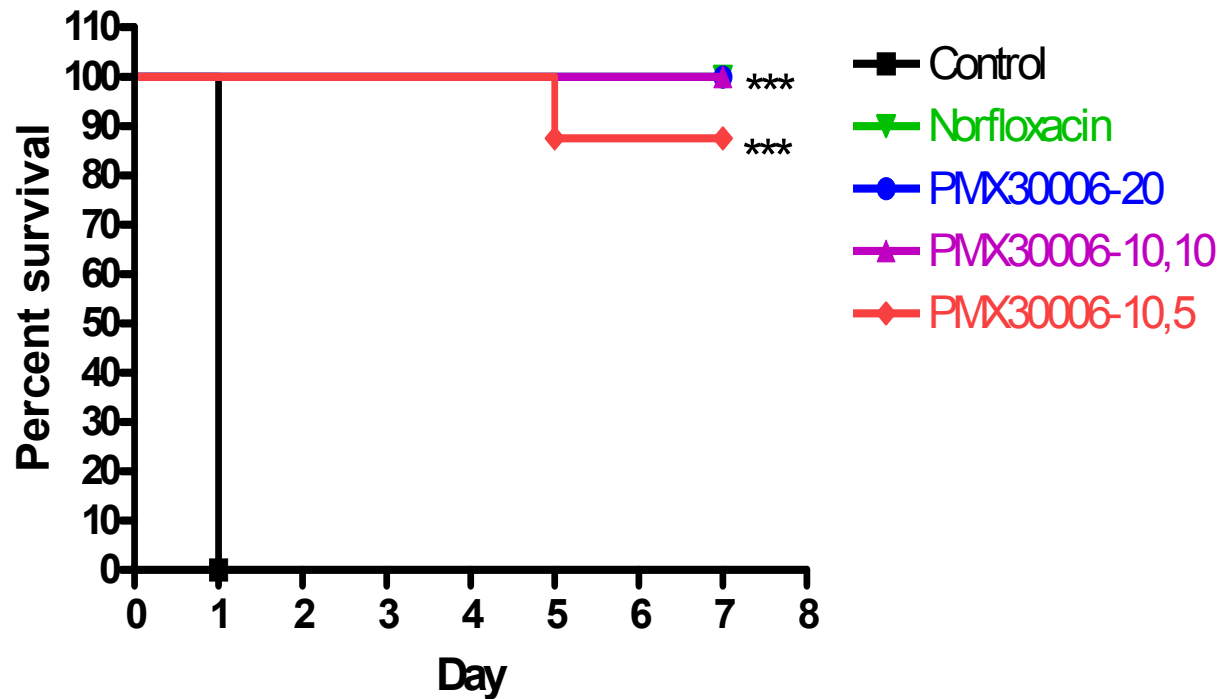
11. Effect of Dosing Interval on Efficacy in the Mouse Thigh Burden Model



Methods: Neutropenic mice were inoculated in the posterior thigh muscles with *S. aureus* ATCC13709 at 1×10^6 cfu/thigh and then treated with PMX30016 or PMX10129 by iv bolus injection in the tail vein at total doses of 2mg/kg or 4 mg/kg split over 2 doses beginning 1 hour after infection. Dosing intervals were 4, 8 or 12 hours (n = 4/group). PMX30016 was formulated in PBS and PMX10129 was formulated in 0.5x PBS. Both compounds were administered in a dose volume of 2 ml/kg. Bacterial quantitations were done 25 hours post infection. Experiments were performed at Ricerca Biosciences.

Results: Previous studies had indicated that efficacy was improved when the total dose was split over 2 or 3 fractional doses with dosing intervals of 4 or 6 hours, respectively. In this study, effects of adjustments on the dosing interval on a 2 fractional dose format were investigated. For PMX30016, maximum efficacy was achieved with a total dose of 4 mg/kg (2 mg/kg/dose) irregardless of dosing interval. At the lower total dose of 2 mg/kg (1 mg/kg/dose), maximum efficacy of $5\log_{10}$ reductions in cfus was achieved at an 8 hour dosing interval. For PMX10129, there is a clear trend in improvement of efficacy with increasing dosing intervals. At a total dose dose of 4 mg/kg (2 mg/kg/dose), maximum efficacy reaches $4.7\log_{10}$ reductions in cfus and at a total dose of 2 mg/kg (1 mg/kg/dose), maximum efficacy reached $3.7\log_{10}$ reductions in cfus. Pharmacokinetic (PK) and pharmacodynamic (PD) relationships are being investigated in ascending dosing studies in the mouse thigh burden model to determine correlations of efficacy with various PK parameters including Cmax, time over MIC, AUC over MIC and time intervals below MIC.

12. Efficacy of PMX30006 in a Mouse Protection Model



Methods: Mice are infected intraperitoneally with *S. aureus* (ATCC 13709) with an 0.5 ml inoculum of 1.25×10^7 cfu/ml. Beginning one hour after inoculation, PMX30006 was administered by iv injection in a tail vein at 20 mg/kg (single dose), 20 mg/kg (2 doses at 1 and 5 hours post inoculation at 10 mg/kg/dose) and 15 mg/kg (2 doses at 1 and 5 hours post inoculation at 10 and 5 mg/kg/dose, respectively). Norfloxacin was used as the positive comparator agent and was administered subcutaneously at 1 hr after inoculation only. Control mice received vehicle only, 1 hour after inoculation. All

animals (n = 8/group) were observed for mortality over seven days following treatment. Experiments were performed at Ricerca Biosciences.

Results: Kaplan-Meier analysis of the survival data is presented. All inoculated mice that did not receive an active agent (control) died within 24 hours whereas all mice that were treated with the positive control agent, norfloxacin (6 mg/kg subcutaneously), survived. Complete protection was also observed with PMX30006 when administered as a single dose (20 mg/kg) one hour after inoculation or as two doses (10 and 10 mg/kg) 1 and 5 hours after inoculation. Only one animal died at day 5 in mice treated with two doses of PMX30006 (10 and 10 mg/kg) 1 and 5 hours after inoculation. The differences in survival between control and treatment groups are highly significant ($P < 0.0001$) whereas the difference between treatment groups is not significant ($P = 0.317$). PMX10066 was tested in this same model and 50% protection over a 7 day period was observed when two doses (10 and 5 mg/kg) were administered 1 and 5 hours post inoculation (not shown).