



Sonstige Antibiotika

Sonstige neue Wirkstoffe

Code	Name	Gruppe	Wirksam gegen	Abstract
--	Capuramycin-Analog	Capuramycin-Analog	Mycobakterien	F1-1162
AFN-1552	FABI-Inhibitor	Enoyl-ACP-Reductase Inhibitoren	MRSA	F1-328 ; F1-329 ; F1-340 ; F1-341
DPD-207	XF Antibacterium	k.A. (topisch)	Staphylokokken	F1-3969
MUT37307	FABI-Inhibitor	Enoyl-ACP-Reductase Inhibitor	MRSA	F1-331 ; F1-332 ; F1-333
LTX109	--	k.A. (topisch)	<i>Pseudomonas</i>	F1-3946
NZ2114	--	Plectasin-Analog	Staphylo-/Streptokokken	F1-3962 ; F1-3963 ; F1-3964 ; F1-3965
PA-824	--	Nitroimidazo-oxazine	Mycobakterien	B-881a
--	Diverse Nanoemulsionen	Nanoemulsionen (topisch)	GP+GN-Bakterien	F1-3944 ; F1-3945
--	Tetramic acid	Topisches Präparat	Staphlokokken	F1-3948
--	Thioridazine	Elektronen-Transport Inhibitor	<i>M. tuberculosis</i>	A-1821

Sonstige neue Wirkstoffe

Code	Name	Gruppe	Wirksam gegen	Abstract
NVC-422	Chlorotaurin	Taurin	Staphylokokken	F1-3940
NXL-103	Streptogramin-Kombination	Streptogramine	Staphylokokken	F1-365
RX100472	Pyrimidinanalog	Methionyl-t-RNA-Synthetase Inhibitor	Gram(+) Bakterien	F1-334 ; F1-335 ; F1-336 ; F1-337 ; F2-2070
SQ641	Capuramycin	Nukleosid	Mycobakterien	C1-3851 ; F1-1163
SQL109		Zellwandsynthese-Inhibitor	Mycobakterien	C1-3848
TMC207		ATP-Synthase-Inhibitor	Mycobakterien	C1-3848 ; B-877
XF-73		Zellmembran-Destruktion	MRSA	F1-3967 ; F1-3968 ; F1-3970 ; F1-2971
BAL30543/ BAL30544/ BAL30545		Dihydrofolat-Reductase Inhibitoren	Gram(+) Bakterien	F1-3936 ; F1-3937 ; F1-3938

A-1821

Thioridazine Has Dramatic Sterilizing Effect Against Extracellular Tubercle Bacilli

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Background: Up to now, no one drug has been able to achieve all three goals of modern anti-TB therapy: a rapid kill of *Mycobacterium tuberculosis* (Mtb) in log-phase growth [bactericidal activity], kill of Mtb under acidic conditions and non-replicating persistent bacilli (NPR) [sterilizing activity], and prevention of resistance. Thioridazine (Thio) inhibits the electron transport chain of Mtb.

Methods: We examined the effect of different concentrations (conc) of Thio against Mtb H37Rv using standard PK-PD methods. The inhibitory sigmoid E_{\max} relationship between Thio conc and kill of log-phase growth Mtb, Mtb at pH 5.8, and NRP (Wayne model) was examined. Thio maximal kill (E_{\max}) was also compared to that of conc achieved by standard doses of either isoniazid (H), rifampin (R), or pyrazinamide (Z). A hollow fiber study (HFS) was then performed to confirm the bactericidal activity of Thio administered daily with a serum half-life of 7.5h.

Results: The MIC was 10 mg/L by agar dilution method, and there were no resistant isolates to 3x MIC in 9 log₁₀ CFU Mtb. For bactericidal activity Thio had an E_{\max} of 3.7 versus 1.8 log CFU/mL for H after 7 days of exposure. In a 28 day experiment of log-phase growth Mtb, the Thio EC₅₀ decreased 2-fold between day 7 and 28. Against Mtb at pH 5.8 Thio had an E_{\max} of 5.1 versus 3.8 log CFU/ml for Z after 21 days exposure. The E_{\max} for NRP was 6.8 for Thio versus 1.5 log CFU/ml for R after 14 days of exposure. p was <0.001 for all regressions. Thio EC₅₀ for log-phase Mtb, Mtb at pH5.8 and in NRP were 26, 10.5, and 1.5 mg/L. Microbial kill increased at conc up to 4-6 x MIC, after which no further kill occurred, consistent with time related pattern of kill. The bactericidal activity was confirmed in HFS of regimens in which time above MIC was >66%.

Conclusions: Thio has dramatic bactericidal and sterilizing effect in vitro, and a high barrier to resistance. The conc needed to achieve this are higher than those achieved in serum of humans. However, Thio is concentrated multiple fold in the lungs, the most common site of TB.

B-877

Activity of TMC207 Against *Mycobacterium avium* In Vitro and in the Mouse Model

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Background: New drugs are needed to treat *Mycobacterium avium* infections in either immunocompetent or immunocompromised patients. With the exception of the newer macrolides, *M. avium* is not susceptible to most of antibiotics. TMC207 (R207910) is a diarylquinoline with a broad antimycobacterial spectrum. The aim of this study is to assess the activity of TMC207 (TMC) against *M. avium* in vitro and in the C57Bl/6J mouse model where it was tested in monotherapy or in combination with clarithromycin (CLA) and/or amikacin (AMK).

Methods: The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of TMC207 against *M. avium* strain 101 were determined in vitro. In vivo, mice were infected intraperitoneally with 2×10^7 *M. avium* strain 101 and treated 4 weeks later with TMC, CLA and AMK given alone or as the following combinations: TMC+CLA, TMC+AMK, CLA+AMK and TMC+CLA+AMK. TMC, CLA and AMK were given at 25, 200 and 150 mg/kg 5 days per week for 4 months. TMC and CLA were given orally and AMK was given subcutaneously. The CFU counts in the spleens were measured on Lowenstein-Jensen medium to prevent carry-over effects.

Results: In vitro, TMC207 displayed a bacteriostatic activity against *M. avium* strain 101 with an MIC of 0.01 and an MBC of >128 mg/l. In vivo, all treated mice were still culture positive after 4 months of treatment. Regimens including AMK had the greatest bactericidal activity (-4.5 log₁₀ CFU), followed by CLA (-2.99 log₁₀ CFU) and TMC (-1.4 log₁₀ CFU). The addition of either CLA or TMC or both to AMK did not improve the activity of AMK ($p > 0.05$). The combination of TMC and CLA displayed important bactericidal activity (-2.9 log₁₀ CFU) but did not improve the activity of CLA alone ($p > 0.05$).

Conclusions: Despite having a similar MIC, the in vivo efficacy of TMC207 against *M. avium* is much less dramatic compared to the efficacy against *Mycobacterium tuberculosis*, underlining the importance of a bactericidal rather than a bacteriostatic activity.

B-881a

Extended Early Bactericidal Activity (EBA) of PA-824, a Novel Drug for Tuberculosis Treatment

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Background: PA-824 is a novel nitroimidazo-oxazine being evaluated for its potential to improve TB therapy. PA-824 has undergone: 1) extensive nonclinical evaluation, which has recently revealed potential ocular and male reproductive toxicities at high doses; 2) Phase I clinical evaluation, and 3) a recently completed Phase IIa, extended EBA study.

Methods: This randomized Phase IIa study was conducted at 2 sites in South Africa to evaluate safety, pharmacokinetics (PK) and extended EBA of PA-824 in drug-sensitive, sputum smear-positive, adult TB patients. 15 patients per cohort received 1 of 4 doses of oral PA-824: 200, 600, 1000 or 1200 mg/day for 14 days. A fifth cohort of 8 subjects received once daily, Rifafour e275 (standard, 4-drug, TB treatment), as control for the laboratory methodology. The primary efficacy endpoint was the fall from Day 0 - 14 in log colony forming units (logCFU)/day/ml sputum of *Mycobacterium tuberculosis* (EBA0-14 ± standard error).

Results: PA-824 appeared safe and well-tolerated. Consistent with its PK in healthy volunteers, the drug demonstrated dose-linear but less than dose-proportional increases in C_{max} and AUC when administered in doses from 200 to 1000 mg daily. 1200 mg daily gave no additional exposure. The mean EBA0-14 of Rifafour was 0.148 (±0.021), consistent with that found in previous studies. The mean EBA0-14 of PA-824 was 0.098 (±0.009), and was equivalent for all four dose groups.

Conclusions: PA-824 demonstrated clinically significant extended EBA over the dose range of 200 - 1200 mg daily. Because maximum efficacy was unexpectedly achieved at the lowest dose tested, the extended EBA of lower doses needs to be explored.

C1-3848

The New Antitubercular Drugs SQ109 and TMC207 Act Synergistically In Vitro to Kill *M. tuberculosis*

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Background: SQ109 and TMC207 are two promising new antitubercular drugs currently in human clinical trials. TMC207 inhibits mycobacterial ATP synthase; SQ109 inhibits mycobacterial cell wall synthesis. Each drug by itself has potent activity against susceptible and MDR tubercle bacilli in vitro and are active in *M. tuberculosis*-infected mice. We investigated interactions of SQ109 and TMC207 in vitro for a variety of antimycobacterial activities.

Methods: *M. tuberculosis* H37RV was used in all studies. Combination drug effects for MIC employed two and three drug combinations in a 96-well microdilution assay. Three assay formats were used for time-to-kill studies: estimation of relative light units (RLU), growth index (BACTEC), and CFU counts. Post antibiotic effects (PAE) were determined by BACTEC. Intracellular killing activity was determined by RLU in J774A.1 mouse macrophage cell line.

Results: The combination SQ109+TMC207 was synergistic (Σ FIC of ≤ 0.5), while TMC207+RIF was additive (Σ FIC 2.0). In three-drug combinations that included RIF at 0.1-0.5 MIC, synergy displayed by SQ109+TMC207 was unchanged. In time-to-kill studies, the combination SQ109+TMC207 was superior to TMC207+RIF and SQ109+RIF by all methods. The PAE following 2 hr exposure to SQ109, TMC207 and SQ109+TMC207 were 0, 9 and 13.6 hr, respectively, compared to 15 hr for INH. Order of intracellular killing activity was SQ109+TMC207 > TMC207+RIF > SQ109+RIF.

Conclusions: SQ109 and TMC207 in combination synergistically enhanced their individual activities against *M. tuberculosis*: MIC of SQ109 in the presence of TMC207 was $\frac{1}{2}$ that of SQ109 alone; MIC of TMC207 in the presence of SQ109 was $\frac{1}{4}$ th that of TMC207 alone. The combination also had an increased rate of killing, PAE and intracellular activity. RIF was synergistic with SQ109 and additive with TMC207. RIF did not affect synergistic interaction and improved MIC of the combination SQ109+TMC207.

C1-3851

Enhancement of Intracellular Activity of Capuramycin (CM) Analogue SQ641 Against *M. tuberculosis* (MTB)

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Background: CM is a nucleoside antibiotic that inhibits peptidoglycan biosynthesis by blocking translocase I enzyme (MraY), an essential enzyme found in all bacteria. CM and its analogues are specifically active against a variety of Mycobacteria, and we identified SQ641 as the most active of the analogues: it is rapidly bactericidal and causes total bacterial disintegration. Despite its potent in vitro activities, SQ641 had only modest intracellular activity against MTB in J774A.1 mouse macrophages.

Methods: To improve intracellular activity, we (a) blocked P-glycoprotein (P-gp) mediated efflux pumps, (b) chemically modified SQ641 structure to avoid P-gp binding, and (c) developed particulate formulations of SQ641 to alter its intracellular location and fate. We tested these variations of SQ641 for activity against MTB in J774A.1 macrophages

Results: Multidrug resistance protein 1 (MDR1) transporter modulators verapamil (VE), cyclosporine (CsA), and d-tocopheryl polyethylene glycol 1000 succinate (TPGS), but not the multidrug resistance associated protein (MRP1) blockers, gemfibrozil and probenecid, caused a dose dependent increase in the activity of SQ641 against MTB, indicating that SQ641 is a P-gp substrate. Conjugation of SQ641 with aminoundecanoic acid also improved its intracellular activity. To evade drug efflux and to deliver drug specifically to macrophages, we entrapped SQ641 in TPGS micelles. SQ641-TPGS-micelles (SQ641-M) were avidly phagocytosed by macrophages, delivering the drug into the phagosomal compartment. SQ641-M caused 5-10 fold increase in intracellular activity compared to free drug, and SQ641-M activity was comparable to INH against MTB in J774A.1 macrophages.

Conclusions: Evasion of drug efflux by blocking P-gp pumps, chemical modification, or entrapment of the drug in TPGS micelles caused significant enhancement in the intracellular activity of SQ641 against MTB.

F1-328

In Vitro Anti-staphylococcal Activity of AFN-1252 with and without Rifampin

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Background: *Staphylococcus aureus* and *S. epidermidis* continue to play an important role in human disease. The emergence of methicillin-resistant staphylococci has complicated the therapy of infections caused by these organisms. New agents are needed to treat these infections. AFN-1252 is a newly developed inhibitor of staphylococcal Fab1 (enoyl-ACP reductase) which is an essential enzyme in the terminal step in the bacterial fatty acid biosynthesis cycle. The purpose of this study was to evaluate the *in vitro* kill extent and kinetics of AFN-1252 alone and in combination with rifampin (RIF) against staphylococci, including methicillin-resistant isolates.

Methods: Two clinical isolates of *S. epidermidis* (MRSE 1 and MRSE 6) and two strains of *S. aureus* (ATCC 29213 and MRSA 1) were studied. MICs were determined using CLSI broth microdilution procedures. Standard time-kill techniques were used at drug concentrations of 4X the MIC with incubation up to 48 hours. All strains were tested with AFN-1252, RIF or AFN-1252 + RIF.

Results: Time-kill kinetics showed that significant regrowth was observed with RIF alone with all strains. With *S. aureus* regrowth started at 3 to 6 hours, and reached no-drug control levels at 24 hours. With *S. epidermidis* regrowth initiation was slower, but cell counts reached no-drug control levels at 48 hours. No regrowth was observed with AFN-1252 alone or in combination with RIF, and kill extent in combination was increased and reached bactericidal levels at 24 - 48 hours in some cases. MICs determined on all culture samples at 48 hours showed that RIF MICs increased from 2 to >32,000 fold in cultures exposed to RIF only, and increased only 0 - 8 fold in cultures exposed to AFN-1252 + RIF. No changes in AFN-1252 MICs were observed in cultures exposed to AFN-1252, RIF or AFN-1252 + RIF.

Conclusions: AFN-1252 significantly reduced the emergence of staphylococcal resistance to rifampin. Further evaluation of combination therapy with AFN-1252 plus RIF would be of interest for therapy of complicated infections due to resistant staphylococcal strains.

F1-329

Efficacy of AFN-1252 and Vancomycin in the Mouse Subcutaneous Abscess Model with a Methicillin-Resistant *S. aureus*

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Background: AFN-1252, a novel antibiotic inhibitor of the bacterial fatty acid biosynthesis (FAS II) pathway, is currently under clinical development as an oral and intravenous agent for susceptible and multi-drug resistant staphylococcal infections. AFN-1252 specifically targets the essential enzyme FabI (enoyl-ACP reductase). The current study was performed to determine the efficacy of AFN-1252 (AFN) and vancomycin (Van) in a mouse model of skin and skin structure MRSA infection.

Methods: Female CD-1 mice were rendered neutropenic by a single IP injection of cyclophosphamide (150 mg/kg) on day -4 prior to infection. Abscesses were formed on the flanks of mice by the subcutaneous injection of 10⁵ CFU of an *S. aureus* (MRSA) culture mixed with Cytodex (dextran) beads. Treatment with AFN (orally) or Van (intraperitoneal) was initiated 2 hrs post-infection and continued for three days either once-per-day (qd) or twice-per-day (bid). Abscesses were removed, homogenized and plated 18 hrs after the last dose. Efficacy was determined as the change in CFU/abscess as compared to vehicle treated controls.

Results: The MICs of AFN and Van for the MRSA strain used in this study were determined to be 0.008 and 1 ug/mL, respectively. Abscesses in vehicle treated control animals exhibited a mean bacterial density of 8.7 log CFU/abscess at the end of the study. AFN, administered orally at 100, 30 or 10 mg/kg, demonstrated log reductions of 5.9, 5.2 and 2.5 CFU when administered bid and 5.2, 4.1 and 2.4 CFU when dosed qd, respectively, as compared to the vehicle treated control animals. Van dosed at 30 mg/kg IP bid exhibited a 4.4 log CFU reduction.

Conclusions: The results of this study demonstrate the *in vivo* efficacy of AFN-1252 in a murine subcutaneous abscess model with an MRSA strain and support its further study and development for both susceptible and resistant *S. aureus* skin infections.

F1-331

MUT37307 A Novel Antibacterial Against Methicillin Resistant Staphylococci

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Background: To determine the *spectrum of activity* of the novel FabI inhibitor against different bacterial species. To evaluate the antistaphylococcal activity of MUT37307 on a large number of coagulase-negative staphylococci and *S.aureus* recent clinical isolates.

Methods: Susceptibility to MUT37307 was determined for a spectrum of microbes and compared to Triclosan. Furthermore MICs were determined for 294 staphylococcus recent isolates using the microbroth dilution method and Linezolid was used as a comparator.

Results: Potent activities (MICs: 0.003-0.5 mg/L) were observed for MUT37307 against the Gram-negative organisms *E. coli* (and ESBL-producing strain), *H. influenzae*, *M. catarrhalis*, *P. vulgaris*. MUT37307 was active against *A. baumannii* and *H.pylori* but inactive against *P. aeruginosa* or the anaerobic bacteria tested.

MUT37307 was highly active against staphylococci including MRSA, CAMRSA (MIC₅₀/MIC₉₀ of 0.03/0.06 mg/L), LRSA and VISA (MICs: 0.03-0.06mg/L). Against MSSA strains MUT37307 showed MIC₅₀/MIC₉₀ of 0.03/0.25 mg/L. MUT37307 was very active against methicillin-resistant *S.hominis* with MIC₅₀/MIC₉₀ of 0.06/0.12 mg/L and methicillin-resistant *S.haemolyticus* strains (MIC₅₀/MIC₉₀ 0.03/0.25 mg/L). MUT37307 was very active against methicillin-resistant *S.epidermidis* (MIC₅₀/MIC₉₀ 0.03/1 mg/L) . Overall MUT37307 was more potent than Linezolid on the staphylococcus strains tested.

Conclusions: The novel compound, MUT37307 has a specific spectrum due to the FabI essentiality in bacterial species. MUT37307 was shown to be very potent against a range of coagulase negative Staphylococci and *S.aureus* recent resistant isolates and represents a good candidate for an antistaphylococcal drug.

F1-332

Comparative Antistaphylococcal Activity of MUT37307

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Background: Methicillin-resistant *S. aureus* strains (MRSA) are problems all over the world, and life-threatening infections caused by community-acquired MRSA strains are increasingly found. Glycopeptide non-susceptible MRSA have also appeared in many locations. MUT37307 is a new FabI lipid synthesis inhibitor under preclinical investigation as a new antistaphylococcal agent. We tested antistaphylococcal activity of MUT37307, vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, levofloxacin, moxifloxacin.

Methods: Of 200 recent MRSA isolates, 127 were community-acquired isolated from sites throughout the US, and 40 hospital-acquired. Strains also comprised 3 hetero-vanco intermediate (hVISA), 25 VISA and 5 vanco-resistant (VRSA). Both hVISAs, 5 VISAs and 1 VRSA were isolated in Hershey. We used agar dilution MICs (CLSI), with added Ca for dapto, 24 h incubation for vanco, and fresh tige powder for each run. Each strain was tested for inducible clindamycin susceptibility by D test.

Results: Twenty-eight strains showed inducible clinda resistance (8 community-, 11 hospital-acquired, 9 vanco non-susceptible). MIC₅₀ and MIC₉₀ values (µg/ml) were: (**siehe nächste Folie**). MUT37307 had excellent anti-staphylococcal activity with an MIC range (µg/ml) of 0.03-0.06, and MIC₅₀ and MIC₉₀ values (µg/ml) both of 0.06, against all strains regardless of phenotype. All strains were susceptible to linez and tige and 21 strains (all VISA) had non-susceptible dapto MICs between 2-4 µg/ml. Quinolone resistance was only found amongst hospital acquired and vanco non-susceptible strains.

Conclusions: MUT37303 had excellent activity against 200 MRSA strains of differing phenotypes, MICs (µg/ml) ranging between 0.03-0.06.

F1-332 (Forts.)

Drug	Community-acquired (127)			Hospital-acquired (40)			hVISA+VISA+VRSA (33)		
	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90
MUT 37303	0.03-0.06	0.06	0.06	0.03-0.06	0.06	0.06	0.03-0.06	0.06	0.06
Vancomycin	0.5-1	1	1	0.25-2	1	1	1->128	4	32
Teicoplanin	0.25-2	1	1	0.25-1	0.5	1	1-.32	8	16
Linezolid	2-4	4	4	1-4	4	4	1-4	2	4
Daptomycin	0.5-1	0.5	1	0.5-1	1	1	1-4	2	4
Tigecycline	0.25-0.25	0.25	0.25	0.125-0.5	0.25	0.5	0.06-1	0.25	0.5
Levofloxacin	0.25-1	0.5	0.5	0.25->16	8	>16	2->16	16	>16
Moxifloxacin	0.06-0.125	0.125	0.125	0.06->32	2	16	0.125-16	8	16

F1-333

MUT37307 FabI Inhibitor: *In Vitro* and *In Vivo* Antibacterial Activity against *S. aureus*

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Background: MUT37307 is a novel antimicrobial agent targeting the essential enzyme FabI. Its antibacterial activities were characterized *in vitro*, time-kill profile, post antibiotic effect (PAE), resistance frequency, cytotoxicity, PK properties and *in vivo* efficacy in the mouse thigh and septicemia infection models.

Methods: Strains used were MSSA and MRSA, Linezolid resistant and VISA strains. Standard procedures were used for the MIC, time-kill, PAE and resistance frequency studies. Single dose PK was conducted on mice by oral, IV and sc administration. *In vivo* assays were performed using the thigh abscess in neutropenic mice and septicemia model of infection in mice.

Results: MUT37307 was highly potent against all *S. aureus* strains tested regardless of the phenotype with a MIC₉₀ of 0.06 µg/mL. Time kill studies showed a time-dependent mechanism of killing and a slow bactericidal effect for all strains tested. Frequencies of resistance were 10⁻⁸ at 4xMIC, and PAE was in the 1H range for the 2 strains tested. MUT37307 did not show any cytotoxicity up to 32 µg/mL in the HepG2 viability assay. In the mouse septicemia model the ED₅₀ of MUT37307 was 6.7 mg/kg (CI_{95%} 4-13.7) against MSSA and 13 mg/kg (CI_{95%} 8-22) for MRSA. At a single dose of 50mg/kg subcutaneous, MUT37307 was as active as Linezolid at 50mg/kg in decreasing MRSA and MSSA infection in the thigh infection model.

Conclusions: MUT37307 is a highly potent antistaphylococcal agent with a new mechanism of action. It was particularly active against methicillin resistant strains *in vitro* and *in vivo*. The mechanism of action results in slow bactericidal activity, a low frequency of resistance at 4xMIC, no cytotoxicity and a good *in vivo* antibacterial activity

F1-334

Discovery and SAR of a Novel Series of Pyrimidine Antibacterials Targeting Methionyl-tRNA Synthase (MetRS)

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Background: Bacterial resistance to current Gram-positive antibacterial agents defines a clear medical need for novel drugs that inhibit novel targets. Previous efforts have demonstrated that potent antibacterial agents can be discovered by targeting methionyl-tRNA synthetase (MetRS) but these agents are tightly serum bound and consequently loss significant potency in serum. Consequently, new antibacterial series targeting MetRS are desired to treat systemic infections.

Methods: Compounds were prepared by synthetic routes that utilize simple pyrimidine starting materials. MICs were determined against *S. aureus*, *S. pneumonia* and *E. faecalis* using CLSI guidelines. Enzymatic MetRS inhibition was determined by measuring the incorporation of radiolabeled methionine to tRNA. Structure Activity Relationships (SAR) were developed and new compounds designed guided by structural information from multiple ligand-protein complexes.

Results: Starting with a virtual screening hit with modest high micromolar activity, a pyrimidine-based series of compounds were designed that are highly potent MetRS inhibitors with good antibacterial activity versus Gram-positive pathogens. Further rounds of structure-based drug design yielded compounds that retain good antibacterial potency with or without serum. Rx100472 is a compound typical of this series: *S. aureus* (Smith) MIC 1 µg/mL (+/- 20% serum); *S. aureus* MetRS IC₅₀ 1.2 nM.

Conclusions: Structure-based drug design yielded a novel highly potent antibacterial series with Gram-positive spectrum. Due to their good antibacterial activity in serum, multiple compounds were selected for further in vitro and in vivo characterization.

F1-335

***In Vitro* Activity of Rx100472 against Clinically Important Bacteria**

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Background: With bacterial drug resistance increasing, new drugs with novel mechanisms of action are urgently needed. Rx100472 is a potent antibacterial compound from a novel pyrimidine series targeting methionyl-tRNA synthetase. We evaluated Rx100472 against various Gram-positive and Gram-negative bacteria, including a large panel of *Streptococcus pneumoniae* strains with varying levels of penicillin resistance.

Methods: Rx100472 was evaluated using the standard CLSI broth microdilution method to determine MIC values for forty-eight different Gram-positive and Gram-negative bacterial strains from ATCC and other sources. MIC values were determined for 109 *S. pneumoniae* clinical isolates (most of US origin) from the following susceptibility groups: 39 penicillin-susceptible (PSSP), 35 penicillin-intermediate (PISP), and 35 penicillin-resistant (PRSP). Comparator agents were vancomycin and trimethoprim.

Results: The initial MIC screen showed Rx100472 to be active only against Gram-positive organisms, with MIC values of 0.06 µg/mL to 2 µg/mL. The exception was poor activity of Rx100472 against two *Bacillus cereus* strains and some of the *S. pneumoniae* strains. Rx100472 and trimethoprim exhibited similar potency against the 35 PSSP isolates tested. There were 15 PISP isolates with trimethoprim MIC values significantly higher than those of Rx100472. Out of 35 PRSP isolates, 28 showed resistance to trimethoprim and only 7 showed resistance to Rx100472.

Conclusions: Rx100472 was a very potent antibacterial agent against most of the Gram-positive organisms tested but lacked potent activity against Gram-negative bacterial species. Potency against *S.pneumoniae* might parallel penicillin-susceptibility.

F1-336

Advanced Microbiology Study of Rx100472, A Novel Methionyl tRNA Synthetase (MetRS) Inhibitor

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Background: Antibiotic resistance in Gram-positive organisms is a growing concern to the medical community, leading to a need for the development of new antimicrobial agents active against novel targets. Investigation of a series of compounds targeting methionyl tRNA synthetase led to the discovery of Rx100472, a novel inhibitor with potential for pharmaceutical applications. Extensive *in vitro* microbiology was assessed.

Methods: The *in vitro* activity of Rx100472 against *S. aureus* was investigated in detail. First, this compound was evaluated using standard CLSI broth microdilution method in Mueller Hinton-CA broth with or without 20% serum. Constant concentration time-kill curves were performed using compound at 2x, 4x, 8x the MIC. Samples were taken at 1, 3, 6, 12 and 24 hours, dilutions plated and CFU per time point determined. Single -step resistance mutation frequencies were obtained for 4x and 8x the MIC of Rx100472 and ciprofloxacin towards *S.aureus*. IC₅₀ and Ki values were established for *S. aureus* MetRS. Finally, the mechanism of action for Rx100472 was confirmed by our antisense hypersensitivity assay.

Results: The MIC value for Rx100472 was 1µg/mL with or without 20% serum. In a time-kill assay, addition of Rx100472 resulted in bacterial growth inhibition by 24 hours. Resistance selection mutation frequencies for Rx100472 ranged from 10⁻⁸ to 10⁻¹¹. The enzyme IC50 and the Ki values were determined as 1.2 nM and 0.03nM respectively. Our mechanism of action assay showed that Rx100472 was specific for MetRS with an 8.5 fold shift in antisense hypersensitivity.

Conclusions: Rx100472 is shown to have a potent antimicrobial profile targeting the methionyl t-RNA synthetase in *S. aureus*.

F1-337

***In Vivo* Efficacy of Rx100472, A Novel Methionyl tRNA Synthetase (MetRS) Inhibitor**

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Background: Methionyl tRNA synthetase is a novel target for antimicrobial drug development. New inhibitors of this target, such as Rx100472, inhibit bacterial protein synthesis resulting in cell death. In this study we evaluated Rx100472 for *in vivo* efficacy in a mouse protection assay.

Methods: Pharmacokinetics (PK) of Rx100472 was evaluated following single dose administration in CD-1 mice. Rx100472 was dosed intravenously (IV) at 1 mg/kg or orally (PO) at 5 mg/kg. PK parameters were determined. *In vivo* efficacy of Rx100472 was assessed against both *Staphylococcus aureus* (ATCC13709) and *Bacillus anthracis* (Ames) infections. Mice were infected intraperitoneally with 3×10^7 cfu of *S. aureus* and then dosed at 0 and 4 hours post infection with Rx100472 at 50, 25, 10, and 5 mg/kg. In a second experiment, the mice were infected intraperitoneally with a vegetative inoculum of *B. anthracis* at a challenge dose of 624 cfu/mouse. Immediately after infection, 50 mg/kg of 100472 was dosed intravenously. Mice received additional doses of Rx100472 at 4 hr and 20 hr post-infection.

Results: PK parameters showed a 36 minute half-life by IV and 44 minute half-life by oral dosing. Rx100472 demonstrated a dose-dependent increase in survival in a lethal *S. aureus* systemic infection model with an EC_{50} of 3.5 mg/kg. In a *B. anthracis* challenge model, Rx100472, was efficacious at 50mg/kg when administered within the first 24 hours of infection.

Conclusions: Data from studies with Rx100472 shows that it is efficacious against *S. aureus* and *B. anthracis* systemic infections. Based on this data, Rx100472 is a potential antibacterial development candidate against Gram-positive organisms

F1-340

Specific Spectrum Activity of AFN-1252 against Bacterial Pathogens Isolated from Patients in Canadian Hospitals: CANWARD 2007

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Background: AFN-1252 (AFN) is a potent inhibitor of enoyl-ACP reductase (FabI) that is being developed as a PO/IV specific spectrum agent for treatment of staphylococcal infections. We determined the in vitro activity of AFN against recent gram-positive (GP) and gram-negative (GN) pathogens isolated from patients in medical and surgical wards, intensive care units, clinics, and emergency rooms at 12 Canadian hospitals from Jan-Dec 2007.

Methods: AST was performed using the CLSI broth microdilution method.

Results: The activity of AFN versus selected comparators is summarized below.

Conclusions: AFN-1252 demonstrated potent activity against staphylococci, including MRSA, MRSE, VISA, and VRSA but was inactive against other GP and GN pathogens. These data support the continued development of AFN-1252 for treatment of resistant staphylococcal infections.

Organism (n)	MIC ₅₀ /MIC ₉₀ (ug/ml)			
	AFN-1252	Cefazolin	Ciprofloxacin	Vancomycin
MSSA (372)	≤0.008/ ≤0.008	£0.5/1	0.5/8	1/1
MRSA (130)	≤0.008/ ≤0.008	64/>128	>16/>16	1/1
MSSE (43)	≤0.008/0.03	1/4	4/>16	1/2
MRSE (9)	≤0.008**	64**	>16**	1**
<i>S. pneumoniae</i> (490)	>4/>4	NA	1/2	≤0.06/0.5
<i>E. faecalis</i> (81)	>4/>4	32/128	2/>16	1/2
<i>E. faecium</i> (38)	>4/>4	>128/>128	>16/>16	0.5/>8
<i>E. coli</i> (599)	>4/>4	2/64	≤0.06/>16	>8/>8
<i>K. pneumoniae</i> (199)	>4/>4	2/8	≤0.06/0.5	>8/>8
<i>P. aeruginosa</i> (137)	>4/>4	>128/>128	0.5/16	>8/>8
*VISA (12)	0.015/0.12	128/>128	>16/>16	4/4
*VRSA (7)	0.015**	128**	>16**	>16**

*Isolates obtained through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program: supported under NIAID, NIH Contract No. N01-AI-95359. **Median MIC.

F1-341

Specific Spectrum Activity of AFN-1252 Against Aerobic and Anaerobic Bacterial Pathogens

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Background: AFN-1252 is a potent inhibitor of staphylococcal FabI (enoyl-ACP reductase [ENR]), an essential enzyme in bacterial fatty acid synthesis, and is being developed as an oral and IV agent for staphylococcal infections. Various bacterial species possessing alternate ENR forms may not be susceptible to inhibition by AFN-1252. To further assess the AFN-1252 spectrum, a phylogenetic analysis of bacterial ENR was performed and *in vitro* activities against staphylococci, anaerobic bacteria, and typical Gram-positive (G+) and Gram-negative (G-) pathogens were determined.

Methods: Phylogenetic trees were constructed with PHYLIP 3.52 using protein sequences from the NCBI refseq database. Bacterial strains for AST included ATCC reference strains and clinical isolates from the Affinium and Micromyx collections. MICs were determined using CLSI methods.

Results: Four distinct bacterial ENR families (FabI, FabL, FabV, FabK) were delineated by phylogenetic analyses. Of the 42 representative aerobic and anaerobic G+ and G- species analyzed, only 18 species had FabI only. The remainder had alternate or more than one form of ENR. AFN-1252 showed highly potent activity against both susceptible and resistant staphylococcal species but no activity against streptococci, enterococci, common G- pathogens and 25 anaerobic G+ and G- species including typical gut bacteria.

Conclusions: The AFN-1252 *in vitro* spectrum of activity was highly correlated with the presence of an essential, single FabI enzyme in a susceptible species such as *Staphylococcus*. Species non-susceptible to AFN-1252 had an alternative ENR enzyme or multiple enzyme forms. The lack of activity against common gut and skin flora highlights the potential safety benefits of AFN-1252 and the possibility that adverse effects associated with antimicrobial therapy such as diarrhea, antibiotic induced colitis, *C. difficile* infections and candidiasis will be much less frequent.

F1-365

Antistaphylococcal Activity of NXL 103 Compared to Other Agents

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Background: MRSA are therapeutic problems all over the world and life-threatening infections caused by community-acquired MRSA strains are increasingly found. MRSA strains not susceptible to glycopeptides have appeared in many locations. NXL 103 is an oral combination of two streptogramins with activity against Gram-pos and -neg aerobic and anaerobic strains. We describe anti-MRSA activity of NXL 103 compared to vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, azithromycin, clarithromycin, clindamycin and quinupristin/dalfopristin.

Methods: 200 MRSA isolates were tested: of these 127 were community-acquired, isolated from sites throughout the US, and 40 were hospital-acquired. Strains also comprised 3 hetero-vanco intermediate (hVISA), 25 VISA and 5 vanco-resistant (VRSA). Both hVISA, 5 VISA and 1 VRSA were isolated in Hershey. Agar dilution MICs, (CLSI), were used with added Ca for dapto, 24 h incubation for vanco, and fresh tige powder for each run. Each strain was tested for inducible clinda S by the D test.

Results: Twenty-eight strains showed inducible clinda R (8 community-, 11 hospital-acquired, 9 vanco non-S). MIC₅₀ and MIC₉₀s (µg/ml) were (**siehe nächste Folie**)

NXL 103 has excellent activity against all strains regardless of phenotype with MICs ranging from 0.125-0.5 µg/ml. and MIC₅₀ and MIC₉₀s of 0.25 and 0.25-0.5 µg/ml, resp, in vanco S strains Against vanco non-S strains NXL 103 had MICs of 0.06-2 µg/ml, with MIC₅₀ and MIC₉₀s of 0.5 and 1 µg/ml. All strains were S to linez, quinu/dalfo, and tige and all except 21 strains (all VISA)(MICs 2-4 µg/ml) were dapto S. All community-acquired and the majority of other strains were macrolide R.

Conclusion: NXL 103 was very active against all MRSA, with an MIC range of 0.06-2 µg/ml.

F1-365 (Forts.)

Drug	Community-acquired			Hospital-acquired			hVISA+VISA+VRSA		
	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90
NXL 103	0.125-0.5	0.25	0.25	0.125-0.5	0.25	0.5	0.06-2	0.5	1
Vanco	0.5-1	1	1	0.5-2	1	1	1->128	4	32
Teico	0.25-2	1	1	0.25-1	0.5	1	1->32	8	16
Linez	2-4	4	4	1-4	4	4	1-4	2	4
Dapto	0.5-1	0.5	1	0.5-1	1	1	1-4	2	4
Tige	0.25-0.25	0.25	0.25	0.125-0.5	0.25	0.5	0.06-1	0.25	0.5
Azithro	>8->8	>8	>8	1->8	>8	>8	1->8	>8	>8
Clarithro	>8->8	>8	>8	0.25->8	>8	>8	0.25->8	>8	>8
Clinda	0.125-0.25	0.25	0.25	0.125->16	0.25	>16	0.06->16	>16	>16
Quinu/Dalfo	0.25-1	0.5	0.5	0.25-1	0.25	1	0.125-2	0.5	1

F1-1162

Activity of SQ641, a Capuramycin Analog, in Murine Models of Tuberculosis (TB)

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Background: Capuramycin analogs SQ997, SQ922, and SQ641 are inhibitors of translocase I (TL-I), an enzyme involved in cell wall synthesis of all bacteria. Each analogue has *in vitro* activity against a broad range of *Mycobacteria*. SQ641 is the most potent of these bactericidal compounds, with *M. tuberculosis* MIC of 4 µg/ml, post antibiotic effect of 55 hr, and bactericidal activity and rate of kill surpassing that of INH and RIF. Cellular drug efflux that affected intracellular accumulation to kill *M. tuberculosis* in macrophages and poor drug solubility meant that *in vivo* activity of the drug did not reflect its potent *in vitro* activities. We evaluated methods to improve the *in vivo* activity of the analogues.

Methods: Two murine models of TB were used: a weight-loss screening assay and the classic chronic infection assay. Mice were infected with *M. tuberculosis* H37RV at doses consistent with the different models. All three drugs were delivered by 4 methods: gavage (po), intravenous (iv), intraperitoneal (ip) or intranasal (in). Drugs were dissolved in water (ip, iv, in) or 5% alcohol (po). SQ641 was also dissolved in 2.5% d-α-Tocopheryl polyethyleneglycol-1000 (TPGS) to enhance its solubility.

Results: All three drugs dissolved in water or alcohol and given for 3 wk of TB therapy by each of the delivery methods reduced viable *M. tuberculosis* less than 1 log₁₀ CFU in lungs: CFU reduction was comparable to that of RIF or INH given in 1/10 optimal dose. SQ641 dissolved in 2.5% TPGS and given ip for 2 wk of TB therapy, however, was more effective than INH: SQ641 reduced bacteria in lung by 1.7 log₁₀CFU, whereas INH reduced bacteria in lung by 1.3 log₁₀ CFU.

Conclusions: Addition of SQ641 to TPGS, routinely used to improve solubility of drugs and which inhibits p-glycoprotein drug efflux in cells, significantly increased the anti-TB efficacy of SQ641 *in vivo*. These studies demonstrated the antitubercular potential of TL-1 inhibitors that may be further improved by additional formulation changes to improve solubility and intracellular activity.

F1-1163

In Vitro Activities of Capuramycin (CM) Analogues against Nontuberculous Mycobacteria (NTM).

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Background: NTM often cause infections in immunocompromised patients, and such infections are on the rise. NTM are generally resistant to conventional anti-TB drugs and there is dearth of effective drugs to treat these infections. CM belongs to a new class of antibiotics that inhibit bacterial cell wall synthesis by blocking translocase I, an essential enzyme in peptidoglycan biosynthesis. CM has a narrow spectrum of activity, with highest activity against the *Mycobacteria*.

Methods: In this study we investigated activities of 11 different CM analogues against three common species of NTM - 10 MAC strains, one each of *M. kansasii* (MKN) and *M. abscessus* (MAB). MIC was determined by microdilution method and synergy between different drugs was determined by checkerboard titration.

Results: The MIC of CM analogues against three different NTMs are shown in the table: **(siehe nächste Folie)**

Conclusions: We selected 4 µg/ml as the highest MIC value that indicated drug activity. Five analogues (SQ641, RKS2033, RKS2235, RKS2244, RKS2243) were active against MAC. Four analogues (SQ641, RKS2033, RKS2241, RKS2244) were active against MAB, and 3 of these (SQ641, RK2033, RK2244) were also active against MAC. Ten of the 11 analogues (except RS120029) were active against MKN. SQ641 and RK2033 have excellent activity against all three NTM; however, SQ641 was the most active against all the NTM tested. Moreover, SQ641 was synergistic with Ethambutol in MAC and with Streptomycin and Rifabutin in MAB.

F1-1163 (Forts.)

Compound	MIC ($\mu\text{g/ml}$) range [MIC ₉₀] MAC	MIC ($\mu\text{g/ml}$) MAB	MIC ($\mu\text{g/ml}$) MKN
SQ997	4 -32 [16]	16	2
SQ922	0.5 - >32 [16]	16	0.5
SQ641	0.125 - 8 [2]	0.5	0.06
RKS2186	8 - 1 [8]	16	2
RKS2033	1 - 8 [4]	2	0.5
RKS2137	0.5 - 16 [8]	8	0.5
RKS2243	0.5 - 8 [4]	>32	0.5
RKS2235	1 - 8 [4]	16	1.0
RKS2244	0.5 - 8 [4]	4	0.25
RKS2241	0.5 - 16 [8]	2	0.5
RS120029	32 - >32 [32]	>32	32

F1-3936

The Comparative Activity of the Dihydrofolate Reductase Inhibitors Bal30543, Bal30544 and Bal30545 Against Clinical Strains of Gram-positive Pathogens from the UK

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Background: BAL30543, BAL30544 and BAL30545 are new dihydrophthalazine inhibitors of dihydrofolate reductase (DHFR with activity against a broad range of Gram-positive bacteria. The dihydrophthalazine substituent confers potent inhibitory activity against the more prevalent staphylococcal DHFR variants that are resistant to trimethoprim. In this study we investigated the activity of these new inhibitors against recent clinical isolates from the UK.

Methods: A total of 225 clinical strains; methicillin sensitive *Staphylococcus aureus* (25), methicillin resistant *Staphylococcus aureus* (25) hVISA (25), VISA (17), coagulase negative staphylococci (25), Beta haemolytic streptococci Group A (17), Beta haemolytic streptococci Group B (13), Beta haemolytic streptococci Group G (12), *Str.pneumoniae* (15), *Corynebacteria* spp. (12), *Listeria monocytogenes* (10) and *Streptococcus milleri* (8) were tested against BAL30543, BAL30544 and BAL30545 and 6 comparators; trimethoprim (TRI), linezolid (LIN), daptomycin (DAP), moxifloxacin (MOX), vancomycin (VAN) and minocycline (MIN). MICs were determined using CLSI methodology, CLSI M7-A6 agar dilution method; using Mueller Hinton agar supplemented with 5% lysed horsed blood, *S.aureus* ATCC 25923 and *Ent. faecalis* ATCC 29212 were used as control strains.

Results: The MIC50s/MIC 90s for BAL30543, BAL30544, BAL30545 and the 6 comparators are shown in the table (**siehe nächste Folie**). Daptomycin was the most potent of the comparator agents tested.

Conclusion: BAL30453, BAL30450 and BAL30455 demonstrated good activity against these clinically important Gram-positive strains. BAL30453 was the most potent of the new compounds.

F1-3936 (Forts.)

Antimicrobial	BAL30543	BAL30544	BAL30545	TRI	LIN	DAP	MOX	VAN	MIN
MSSA	0.03/0.03	0.03/0.03	0.12/0.25	1/2	2/2	0.25/0.25	0.12/0.25	1/1	0.25/0.25
MRSA	0.015/0.06	0.06/0.25	0.06/0.06	0.25/32	2/2	0.12/0.25	4/8	1/1	0.25/0.25
hVISA	0.03/0.06	0.25/0.25	0.06/0.06	1/16	1/2	0.5/0.5	4/4	2/4	0.25/0.5
VISA	0.03/0.25	0.25/0.5	0.06/0.5	1/32	1/2	1/2	8/8	4/8	1/4
CNS	0.06/8	0.06/8	0.06/4	16/32	1/2	0.25/0.5	0.25/8	1/2	0.5/2
BHSA	0.06/0.06	0.06/0.06	0.06/0.12	0.5/1	1/1	0.015/0.3	0.25/0.5	0.25/0.25	0.12/0.25
BHSB	0.03/0.06	0.06/0.12	0.06/0.12	1/1	1/2	0.015/0.06	0.25/0.5	0.25/0.25	0.25/16
BHSC	0.015/0.06	0.06/0.06	0.006/0.12	0.5/1	1/2	0.03/0.12	0.25/0.5	0.12/0.5	0.25/4
BHSG	0.03/0.06	0.06/0.06	0.03/0.06	1/1	2/2	0.015/0.03	0.25/0.5	0.12/0.12	0.25/16
STRPN	0.06/0.12	0.12/0.25	0.12/0.12	1/4	1/2	0.06/0.12	0.25/0.25	0.12/0.25	0.25/0.25
CORYNE	0.06/4	0.12/4	0.12/4	16/16	0.5/0.5	0.06/0.12	1/8	0.25/0.5	2/16
LISMO	0.015/0.015	0.03/0.03	0.06/0.06	0.06/0.06	2/2	1/2	0.5/1	1/1	0.25/16
STRMI	<0.008	0.03	0.015	4	1	0.25	0.25	1	0.25

F1-3937

Antistaphylococcal Activities of Dihydrophthalazine Dihydrofolates, a Family of Novel Antibacterial Drugs, by Time-Kill

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Introduction: Methi-S and -R staph are increasing, and community MRSA causing severe infections spreading. Staph with decreased glycopeptide S have emerged, so other drugs are needed. Dihydrofolate reductase (DHFR) is a confirmed drug target, but activities of marketed DHFR inhibitors inadequately cover staph or enterococci. BAL0030543, BAL0030544, BAL0030545 are novel dihydrophthalazine antifolates with potent antistaph and antienteroc activity.

Methods: Time-kills were done for 6 *S. aureus* [2 MSSA, 4 MRSA; 2 VISA, 1 VRSA; 3 trimethoprim- R (Tmp^R)] and 6 coagulase-neg staph [2 MSCoNS, 4 MRCoNS; 1 VCoNS, 1 linezolid-R (Lzd^R), 4 Tmp^R] in MHB (Oxoid; 5x10⁵-5x10⁶ CFU/ml), overnight incub in a shaking water bath (35°C); viabilities were at 0, 3, 6, 12, 24 h. Drugs (BAL0030543, BAL0030544, BAL0030545; Tmp; Lzd; minocycline, Min; clindamycin, Cli; vancomycin, Van; rifampin, Rif) were tested at 1, 2, 4 x MIC.

Results: MICs (µg/ml) {n} were: BAL0030543 {12}, 0.008-8; BAL0030544 {12} and BAL0030545 {12}, 0.03-4; Tmp {5}, 0.5->128; Lzd {11}, 1-64; Min {12}, 0.125-4; Cli {7}, 0.125->128; Van {11}, 1->256; Rif {11}, 0.008->128. Time-kills were not done when MICs were ³64 µg/ml. No. strains with 90%, 99%, 99.9% killing at 1xMIC and 2xMIC were: (**siehe nächste Folie**).

By 24 h at 2xMIC, BAL0030543, BAL0030544, and BAL0030545 had 99.9% killing against 9-11 of 12 strains at 2xMIC; Tmp was cidal for 3 of 5 strains tested; Van was cidal for 7 of 11 strains tested; and Rif was cidal for 2 of 11 strains tested; but Min, Cli, and Lzd were static by 24 h.

Conclusions: BAL0030543, BAL0030544, BAL0030545 had low MICs and excellent killing by 24 h towards a variety of *S. aureus* and CoNS strains.

F1-3937 (Forts.)

Antibiotic (multiplicity of MIC)	3 h			6 h			12 h			24 h		
	% killing			% killing			% killing			% killing		
	90	99	99.9	90	99	99.9	90	99	99.9	90	99	99.9
BAL0030543												
2 x MIC	4	0	0	12	6	0	12	12	4	12	12	11
1 x MIC	3	0	0	12	4	0	12	8	3	8	6	6
BAL0030544												
2 x MIC	5	1	0	12	7	2	12	12	8	11	10	9
1 x MIC	3	1	0	11	7	2	11	8	4	8	6	3
BAL0030545												
2 x MIC	3	0	0	12	8	2	12	12	6	11	11	10
1 x MIC	3	0	0	10	4	1	10	8	4	9	6	5
Trimethoprim												
2 x MIC	3	0	0	5	3	0	5	5	3	5	5	3
1 x MIC	2	0	0	5	2	0	5	4	3	3	2	2
Linezolid												
2 x MIC	0	0	0	1	0	0	4	0	0	8	1	0
1 x MIC	0	0	0	0	0	0	2	0	0	2	0	0
Minocycline												
2 x MIC	1	0	0	3	0	0	4	0	0	7	2	0
1 x MIC	0	0	0	2	0	0	1	0	0	4	0	0
Clindamycin												
2 x MIC	0	0	0	0	0	0	3	0	0	7	3	0
1 x MIC	0	0	0	0	0	0	2	0	0	5	0	0
Vancomycin												
2 x MIC	5	0	0	9	5	2	10	7	3	10	8	7
1 x MIC	5	0	0	8	5	1	8	5	2	8	6	6
Rifampin												
2 x MIC	5	3	1	9	3	1	10	5	1	10	8	2
1 x MIC	4	0	0	8	1	0	9	2	0	5	3	1

F1-3938

Dihydrophthalazine Antifolates, a Family of Novel Antibacterial Drugs: In Vitro Activities against Coagulase-Negative Staphylococci

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Background: Coagulase-negative staphylococci (CoNS) are increasingly implicated in serious systemic infections, particularly in immunocompromised hosts. Most CoNS are now methicillin-resistant (Met^R), and both vancomycin (Van) non-susceptibility and linezolid (Lzd) resistance amongst CoNS are on the rise. Therefore, alternative therapeutic modalities are needed urgently. Dihydrofolate reductase (DHFR) is an established antimicrobial target, but the activities of currently marketed DHFR inhibitors do not provide adequate coverage of staphylococci or enterococci. BAL0030543, BAL0030544, and BAL0030545 are novel dihydrophthalazine derivatives with potent *in vitro* and *in vivo* antistaphylococcal and antienterococcal activities.

Methods: MICs were obtained using Mueller-Hinton agar (Oxoid; 10⁴ CFU/spot as per CLSI guidelines) for 160 unspiciated clinical isolates of CoNS [including 4 Van-intermediate (VI) strains, 1 heterogeneous VI (hVI) strain, and 7 Lzd^R strains].

Results: *MIC*₅₀/*MIC*₉₀ (or geometric mean MIC*) values and {MIC ranges} (μg/ml) for BAL0030543, BAL0030544, BAL0030545 and comparators (trimethoprim, Tmp; Lzd; minocycline, Min; clindamycin, Cli; Van; rifampin, Rif) were as follows (**siehe nächste Folie**)

Conclusions: Antifolates BAL0030543, BAL0030544, and BAL0030545 were highly active against most coagulase-negative staphylococci surveyed, with 93.1-98.1% of strains susceptible to all 3 dihydrophthalazine antifolates at MICs ≤0.5 μg/ml.

F1-3938 (Forts.)

Antibiotic	Met ^S (25)	Met ^R (135)	Tmp ^S (58)	Tmp ^R (102)	VI + hVI (5)*	Lzd ^R (7)*	All CoNS (160)
543	0.016/0.06 {≤0.008-2}	0.06/0.5 {≤0.008->32}	0.016/0.03 {≤ 0.008-0.12}	0.12/0.5 {0.016->32}	0.49 {0.12->32}	0.10 {0.06-0.12}	0.06/0.5 {≤ 0.008->32}
544	0.016/0.03 {≤ 0.008-2}	0.06/0.5 {≤ 0.008->32}	0.016/0.03 {≤ 0.008-0.12}	0.12/0.5 {0.016->32}	0.49 {0.12->32}	0.11 {0.06-0.12}	0.06/0.5 {≤ 0.008->32}
545	0.03/0.06 {≤ 0.008-4}	0.06/0.5 {≤ 0.008->32}	0.03/0.06 {≤ 0.008-0.12}	0.12/1 {0.03->32}	0.87 {0.25->32}	0.11 {0.06-0.12}	0.06/0.5 {≤ 0.008->32}
Tmp	0.25/64 {0.06-128}	128/>128 {0.03->128}	0.25/2 {0.03-4}	>128/>128 {16->128}	>128 {>128}	>128 {>128}	64/>128 {0.03->128}
Lzd	1/2 {1-2}	1/2 {1->16}	1/2 {1-2}	1/2 {1->16}	1 {1}	29 {16->16}	1/2 {1->16}
Min	0.12/0.5 {0.12-0.5}	0.25/1 {0.06-16}	0.12/1 {0.06-16}	0.5/0.5 {0.06-2}	0.32 {0.12-2}	0.5 {0.5}	0.25/0.5 {0.06-16}
Cli	0.12/0.12 {0.12}	0.25/>32 {≤ 0.06->32}	0.12/>32 {≤ 0.06->32}	2/>32 {0.12->32}	5.19 {0.12->32}	1.49 {1-2}	0.12/>32 {≤ 0.06->32}
Van	1/2 {0.5-2}	2/2 {0.5-8}	1/2 {0.5-2}	2/2 {1-8}	4.59 {2-8}	2 {2}	2/2 {0.5-8}
Rif	≤ 0.004/0.016 {≤ 0.004-0.016}	0.008/2 {≤ 0.004->16}	0.008/0.016 {≤ 0.004->16}	0.008/>16 {≤ 0.004->16}	0.011 {0.008-0.016}	0.005 {≤ 0.004-0.008}	0.008/1 {≤ 0.004->16}

F1-3940

Chemical and Biological Properties of NVC-422, a Novel, Stable *N*-Chlorotaurine Derivative

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Background: *N*-Chloramines of the non-essential amino acid taurine play an important role in human innate immunity, but their chemical instabilities (Olszowski et al. 2002. *Amino Acids* 22:145-153) prevent their use as therapeutic agents. In the present study, *N,N*-dichloro-2,2-dimethyltaurine (NVC-422), in which both hydrogens on the β -carbon of taurine are substituted by methyl groups, was synthesized and examined for its chemical stability and biological properties.

Methods: NVC-422 was synthesized by the reaction of chlorine gas with 2,2-dimethyltaurine and isolated as a crystalline solid, or prepared in solution by the reaction of hypochlorous acid with 2,2-dimethyltaurine. The concentration and stability of NVC-422 in solution was determined by UV-visible spectrophotometry. Minimum Bactericidal Concentration (MBC) and time kill (TK) values for *Escherichia coli* and *Staphylococcus aureus* were determined at different pHs (pH range: 4.0-7.5) using modified CLSI methods.

Results: The purity of NVC-422 was determined to be at least 97%. The compound was stable at room temperature in solution at pH 2.6 - 7.1 for over one year. Antibacterial activity against *E. coli* and *S. aureus* was pH dependent, MBCs being lowest at pH 4.0 (2 $\mu\text{g}/\text{mL}$ for *E. coli* and 1 $\mu\text{g}/\text{mL}$ for *S. aureus*). NVC-422 was rapidly cidal, but not lytic, causing a 3-log drop in *S. aureus* CFUs in under 15 minutes, similar to the (unstable) natural product *N, N*-dichlorotaurine.

Conclusions: NVC-422 has greatly improved stability over *N, N*-dichlorotaurine while retaining *N, N*-dichlorotaurine's antimicrobial activity. NVC-422 represents a promising compound for further development as an antiinfective agent.

F1-3944

Novel Nanoemulsion Antimicrobials Tested Against Nine Gram-Positive Species

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Background: Nanoemulsions (NEs) are oil-in-water emulsions composed of pharmaceutically approved substances in nanodroplets that have an average diameter of 180 nm. Previous work has shown that NEs penetrate through hair follicles and skin pores to significant levels in both the epidermis and dermis. Three NEs were evaluated against 9 Gram-positive species.

Methods: MICs (broth microdilution) and MBCs were determined using CLSI standard methods. **(siehe nächste Folie)**

Results: W₂₀5EC, P₄₀₇5EC, and W₂₀5GBA₂ED had MIC₉₀ ranges to ³20 isolates of each species of 1-16, 2-16, and 4-16 µg/ml, respectively, including organisms that were resistant to topical and systemic antimicrobics. MBCs were performed for 35 isolates: W₂₀5GBA₂ED was cidal against all isolates while W₂₀5EC and P₄₀₇5EC were cidal against 94% of isolates.

Conclusions: The antimicrobial spectrum and potency of NEs, and their ability to permeate epidermal and dermal tissues, make them ideal candidates for treatment of superficial skin and soft tissue infections caused by leading Gram-positive pathogens, including MRSA.

F1-3944 (Forts.)

Compound	MIC ₉₀ (µg/ml)								
	<i>S. aureus</i> (38) ^a	<i>S. epidermis</i> (20)	<i>S. hemolyticus</i> (20)	<i>E. faecalis</i> (21)	<i>E. faecium</i> (21)	<i>S. pyogenes</i> (21)	<i>S. agalactiae</i> (20)	<i>S. mitis</i> (21)	<i>S. sanguis</i> (20)
W ₂₀ 5EC	4	2	4	1	4	2	2	16	16
P ₄₀₇ 5EC	4	4	8	4	8	4	2	16	16
W ₂₀ 5G BA ₂ ED	4	4	4	4	4	4	4	16	8
Oxa/Amp/Pen ^b	>2	>2	>2	≤ 8	>16	≤ 0.12	≤ 0.12	>2	1
Erythromycin	>2	>2	>2	>2	>2	≤ 0.25	>2	>2	>2
Clindamycin	>2	>2	>2	>2	>2	≤ 0.25	>2	≤ 0.25	≤ 0.25
Levofloxacin	>4	>4	>4	>4	>4	≤ 0.5	1	2	1
Tetracycline	>8	≤ 2	>8	>8	>8	≤ 2	>8	>8	>8
Trimethoprim/ Sulfamethoxazole	≤ 0.5	>2	>2	>2	>2	≤ 0.5	≤ 0.5	2	2
Fusidic Acid	0.5	0.5	0.25	8	8	8	16	32	32
Mupirocin	≤ 4	>256	>8	256	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
Linezolid	2	1	1	2	2	1	1	1	1
Vancomycin	2	2	2	2	>16	0.5	0.5	0.5	0.5

F1-3945

Antimicrobial Activity of Nanoemulsions Tested Against Seven Gram-Negative Species

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Background: Nanoemulsions (NEs) are composed of pharmaceutically approved substances emulsified to nanodroplets with an average diameter of 180 nm. Previous studies have shown that NEs significantly penetrate the skin or mucosa into underlying tissues without any signs of inflammation. Three NEs were evaluated against 7 Gram-negative species. Many of these species, especially *Pseudomonas aeruginosa* and *Burkholderia cepacia*, are problematic in cystic fibrosis patients.

Methods: MICs and MBCs were determined using CLSI standard methods. MICs to NEs were determined in the presence of 5 mM EDTA, a known enhancer of NE activity. The addition of alamar blue, a redox indicator that yields a colorimetric change in response to metabolic activity, was used to determine the MICs of NEs that are opaque at higher concentrations.

Results: All 3 NEs had activity against Gram-negative isolates (EDTA alone inhibited 3 and 5 strains of *A. baumannii* and *S. maltophilia*, respectively). This included isolates that were resistant to comparator agents. MBCs were performed for 25 isolates; W₂₀5EC, P₄₀₇5EC, and W₂₀5GBA₂ED were bactericidal against 88, 96, and 92% of the isolates, respectively. (siehe nächste Folie)

Conclusions: NEs were broadly active against Gram-negative species, including multidrug-resistant isolates. The documented MICs were within the range of concentrations achievable with topical application to skin or mucosal tissues. One or more of the nanoemulsions may be useful for prophylaxis treatment of chronic pulmonary infections in cystic fibrosis patients.

F1-3945 (Forts.)

Compound	MIC range (µg/ml) for 5 isolates/species						
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>S. maltophilia</i>	<i>B. cepacia</i>
W₂₀5EC	1-2	4-16	4-8	16-32	≤ 0.12-2	≤ 0.12	64->256
P₄₀₇5EC	4	8-16	8-16	32-64	≤ 0.12-4	≤ 0.12	128->256
W₂₀5G BA₂ ED	2-4	4-8	4-16	8-16	≤ 0.12-4	≤ 0.12	32->256
Ceftazidime	≤ 1->16	≤ 1->16	≤ 1	2-4	≤ 1->16	2->16	2-4
Cefepime	≤ 0.12->16	≤ 0.12->16	≤ 0.12	1-8	≤ 0.12->16	4->16	4-16
Piperacillin/ tazobactam	1-8	1-64	≤ 0.5-1	2-16	≤ 0.5->64	8->64	2-32
Imipenem	≤ 0.12-0.5	≤ 0.12-0.25	≤ 0.12-2	1->8	≤ 0.12->8	4->8	4-8
Tobramycin	0.5-16	0.25-16	0.25-1	0.25-1	0.25->16	0.5->16	>16
Levofloxacin	0.03->8	0.06-8	0.06-2	0.25-4	0.06->8	0.5-8	1-2
Colistin	≤ 0.5	£0.5	>4	≤0.5-2	£0.5-2	£0.5-1	>4

F1-3946

The Activity of LTX109 Against Antibacterial-Resistant Clinical Isolates of *Pseudomonas aeruginosa*

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Background: LTX109 is a novel antimicrobial currently being developed as a topical agent for the treatment of skin infections. It is a broad spectrum agent with activity against several important bacteria and fungi. We wished to study the *in vitro* activity against clinical isolates of *Pseudomonas aeruginosa*.

Methods: Isolates (110 in total) were taken from a year 2003 collection of *Pseudomonas aeruginosa* strains stored at Quotient Bioresearch Ltd. MICs were determined using the microbroth dilution method for antimicrobial susceptibility testing published by the Clinical and Laboratory Standards Institute. MIC estimations were performed using wet plates, containing the antibacterials, prepared at Quotient Bioresearch Ltd. Following normal practice all the plates containing Mueller-Hinton broth were prepared in advance, frozen at -70°C on the day of preparation and defrosted on the day of use. Susceptibility to comparator agents was determined using current CLSI breakpoints.

Results: LTX109 showed a mode MIC value of 8 mg/L, with 98% of all isolates having an MIC of 8 or 16 mg/L. Many of the isolates showed resistance to the active comparator agents, ceftazidime, ciprofloxacin, imipenem, gentamicin and sometimes against multiple of these. Resistance to these agents did not affect the activity of LTX109 against *P. aeruginosa*.

Conclusions: LTX109 may be a valuable new agent for treating infections, such as burn wounds, caused by *Pseudomonas aeruginosa*. Further investigations are warranted.

F1-3948

Novel Tetramic Acids as Treatment Candidates for Staphylococcal Infections

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Background: Infections caused by MRSA continue to fuel the need for new therapeutics. In an effort to develop novel antibiotics, we reported the synthesis of a panel of tetramic acid (TA) compounds that exhibit activity against Gram positive bacteria, mainly *S. aureus*. Typically, TAs possess a 2,4-pyrrolidinedione core and are found in many pharmacologically active natural products including the antibiotic reutericyclin, a membrane depolarizing agent. TAs however have not been used to treat MRSA and other bacterial infections. We therefore sought to characterise the activity of TAs against *S. aureus*.

Methods: Five structurally related TAs were studied. MICs were done by NCCLS methods. MBICs and MBECs were determined against biofilms of *S. epidermidis* RP62A, MRSA ATCC 35391 and MSSA ATCC 25293 using the Calgary Biofilm Device. Time kill and macromolecular labelling studies were conducted and effects of TAs on membrane integrity assessed by propidium iodide (PI) uptake and haemolytic assays.

Results: All five TAs were active against MSSA and MRSA clinical isolates (n = 310) with MICs of 0.2-3.12 mg/ml. Activity was also shown against biofilms (MBICs = 0.4-1.6 mg/ml). Interestingly, MBECs of 6.2-200 mg/ml displayed by the four most active TAs were comparable to rifampin and superior to vancomycin (> 400 mg/ml). Exposure of log phase *S. aureus* 8325 to TAs for 6 h resulted in bacteriostasis up to 32 \times MICs. Primarily, RNA and protein synthesis were affected. Though TAs caused haemolysis this action did not result in *S. aureus* cell lysis as shown by PI uptake and turbidimetric assays.

Conclusions: Our representative TAs possess a mode of action that result in good activity against both planktonic and biofilm staphylococcal cells. Although cellular lysis was not detected in *S. aureus*, membrane damage to erythrocytes implies that bacterial membranes are the target for these agents. Studies are ongoing to further characterise the mode of action of TAs and evaluate their potential as topical antistaphylococcal agents.

F1-3962

Activity of NZ2114 against Staphylococcal and Streptococcal Isolates, Including Resistant Phenotypes

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Background: NZ2114 is a plectasin analogue active against Gram-positive (GP) cocci currently being developed for the potential treatment of sepsis, upper respiratory infections, skin and soft tissue infections, pneumonia, and tonsillitis. Staphylococci (STA) and streptococci (STR) are major pathogens of these types of infections. The activity of NZ2114 was evaluated against these organisms, including isolates with clinically relevant resistance.

Methods: Clinical isolates including 121 *Staphylococcus aureus* (SA), 50 coagulase-negative staphylococci (CoNS), 50 *Streptococcus pneumoniae* (SP), 51 Group C and G streptococci (GCG), 45 *S. agalactiae* (SAG), and 50 *S. pyogenes* (SPY) were centrally tested by broth microdilution (CLSI M7-A7) against NZ2114 and comparators. Isolates resistant (R) or non-susceptible (NS) to oxacillin (OX), penicillin (PEN), or a macrolide (MAC) were included. Additional SA NS to vancomycin (VAN), linezolid (LZD), and/or daptomycin (DAP) from the NARSA or Eurofins repository were also analyzed.

Results: (see table)

Conclusions: NZ2114 displayed potent *in vitro* activity against both STA and STR. Based on MIC₅₀/MIC₉₀, NZ2114 had similar activity (within one doubling dilution) against OX-R STA and PEN-R/MAC-NS STR populations relative to susceptible populations. Activity was also apparent against LZD/DAP/VAN-NS populations of SA, though NZ2114 MICs tended to be slightly higher against the evaluated VISA. This activity profile highlights the potential of NZ2114 for infections where STR and STA are commonly encountered.

Organism	Phenotype	Total n	MIC (mg/mL)			
			Range	Mode	MIC50	MIC90
SA	OX S	27	0.5-4	2	2	4
	OX R	94	0.12-32	2	2	4
	DAP NS	8	1-8	2	NA	NA
	LZD NS	13	1-8	2	2	4
	VISA	9	4-64	16	NA	NA
	VRSA	2	2-4	NA	NA	NA
	CoNS	OX S	20	0.12-16	2	2
SP	OX R	30	0.06-16	2	2	4
	PEN S	25	≤0.03-4	1	1	2
	PEN I	10	≤ 0.03-4	1	1	2
GCG	PEN R	15	≤ 0.03-4	1	1	2
	MAC S	34	0.06-8	0.5	1	4
	MAC NS	17	0.25-8	1	1	8
SAG	MAC S	19	0.12-1	0.25	0.25	1
	MAC NS	26	≤ 0.03-1	0.5	0.25	0.5
SPY	MAC S	19	≤ 0.03-8	0.25	0.25	0.25
	MAC NS	28	≤ 0.03-4	0.12	0.12	0.5

F1-3963

Minimum Bactericidal Concentration (MBC) Analysis and Timekill Kinetic (TK) Analysis of NZ2114 against Staphylococci and Streptococci

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Background: NZ2114, a plectasin derivative, is a novel antibiotic with activity against Gram-positive (GP) pathogens. To understand the mode of action of NZ2114, MBC and TK of NZ2114 was evaluated against staphylococci (STA) and streptococci (STR) including clinically relevant resistant (R) phenotypes.

Methods: The MIC and MBC of NZ2114 against 9 *S. aureus* (SA), 7 coagulase-negative STA (CNS), 5 *S. pneumoniae*, and 10 β -hemolytic STR with relevant R phenotypes was determined (CLSI M7-A7; CLSI M26-A; MBC defined as the concentration where a ³ 3-log₁₀ kill was observed). TK of NZ2114 were evaluated at 2X, 4X, and 8X the MIC against 7 SA (including methicillin-R clones [e.g. USA300/100] and vancomycin non-susceptible isolates), 1 methicillin-R *S. epidermidis* (SE), and ATCC strains of *S. pneumoniae* and *S. pyogenes*. Viability was assessed periodically over a 24 hour period and cidal activity was defined as ³ 3-log₁₀ reduction in CFU at 24 hr (CLSI M26-A).

Results: NZ2114 MBC:MIC ratios ranged from 1-2 against all STA and STR regardless of phenotype, excluding 1 penicillin-R *S. pneumoniae* (MBC:MIC ratio >4). Cidality of NZ2114 against SA (including MRSA, VISA/VRSA) occurred within 2-6 hr at concentrations at 4X the MIC and 2X the MIC in some instances, with the exception of the tested VISA where cidality was observed only at 8X the MIC. Cidality of NZ2114 was also observed against methicillin-R SE at 2X the MIC (as early as 3 hr), and against *S. pneumoniae* and *S. pyogenes* at 4X and 2X the MIC, respectively, with a 3-log kill as early as 1 hr.

Conclusions: NZ2114 was cidal by MBC (MBC:MIC ratios of ≥ 2) against STA and STR regardless of resistance. This cidality was validated by TK which included a variety of prevalent R clones of SA. The rapid bactericidal activity of NZ2114 against STR and STA, including prevalent R isolates, highlights the potential of NZ2114 as a cidal agent for the treatment of GP infections involving STR and STA.

F1-3964

In Vivo Pharmacodynamic (PD) Activity of the Plectasin Derivative, NZ2114 (NZ) Against Gram Positive Bacteria in a Murine Thigh Infection Model

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Background: NZ is a novel plectasin peptide antibiotic with activity against gram-positive bacteria, including multiply resistant strains. We used the murine thigh model to characterize the in vivo activity of NZ against *S. pneumoniae* (SP) and *S. aureus* (SA).

Methods: Mice had $10^{6.5-7.6}$ cfu/thigh or lung of either SP or SA when treated with either single doses (10-160 mg/kg) or 5 dose levels of NZ ranging from 0.019-640 mg/kg/24 h fractionated into 1, 2, 4, or 6 doses. The impact of infection site was examined using the lung and thigh model. An Emax model was used to examine the relationship between the PD parameters and efficacy.

Results: PK studies demonstrated a AUC/dose values ranging from 1.3-1.9 and half-lives of 0.38-1 h. Single dose time kill studies demonstrated concentration dependent killing over the dose range (0.5-3.7 log₁₀ cfu/thigh) and prolonged PAEs (3-15 h) against both SA and SP. Efficacy of NZ was similar among dosing intervals and regression with the 24h AUC/MIC indice was strong (R² 0.90) for both SA and SP. The Cmax/MIC indice regression was also strong for SP (R² 0.96). The 24h AUC/MIC needed for a SD and 1 log reduction with each organism group is shown below. Treatment against *S. pneumoniae* required less drug in relationship to the MIC than against *S. aureus*. Drug resistance against other antimicrobial classes did not impact the magnitude of the PD target required for efficacy.

Conclusions: NZ demonstrated concentration-dependent killing and prolonged PAEs against both SP and SP. In vivo activity of NZ was dependent on the AUC of exposure. Drug resistance to other antibiotics did not impact the PD target for NZ. The PD target in this model should be considered in design of clinical trails with this new drug.

Organisms	24h AUC/MIC range (mean ± SE)	
	SD	1 log kill
<i>S. pneumoniae</i> (6)	5-21 (12 ± 7)	6.2-36 (20 ± 12)
<i>S. aureus</i> (8)	7.4-37 (29 ± 11)	18-69 (48 ± 18)

F1-3965

High CSF Penetration and Bactericidal Activity of Plectasin Variant NZ2114, a Novel Antimicrobial Peptide

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Background: Plectasin is the first defensin-type antimicrobial peptide isolated from a fungus and has potent activity against Gram-positive bacteria; however pk/pd properties of the plectasin variant NZ2114 in local infections still remain to be defined.

Methods: Using a rabbit meningitis model, the CSF penetration and bactericidal activity of NZ2114 and ceftriaxone against a penicillin-resistant *Streptococcus pneumoniae* (NZ2114 and ceftriaxone MICs: 0.25 and 0.5 µg/ml, respectively) was studied.

Results: Pharmacokinetics: There was a significant higher CSF penetration of NZ2114 through inflamed - compared to noninflamed meninges ($AUC_{CSF/serum}$: 32% vs. 2%, respectively). CSF conc. peaked at ~3 hours after an iv. infusion of either 20 or 40 mg/kg and remained above 10 x the MIC during a 6-hour study period. Bactericidal activity: Treatment with NZ2114 (40 and 20 mg/kg at 0 and 5 hours, respectively, n=11) caused a significantly higher reduction in CSF bacterial concentrations ($\Delta \text{Log}_{10} \text{CFU/mL}$) as compared to therapy with ceftriaxone (125 mg/kg at 0 hours, n=7) at 3 hours (median: 3.7 (interquartile range: 2.5-4.6) vs. 2.1 (1.7-2.6), respectively, Mann Whitney test, $P=0.001$), at 5 hours (5.2 (3.6-6.1) vs. 3.1 (2.6-3.7), respectively, $P=0.01$) and at 10 hours (5.6 (5.2-5.9) vs. 4.2 (3.6-5.0), respectively, $P=0.03$) after start of therapy as well as compared to untreated meningitis rabbits (n=7, $P<0.05$, data not shown). Also, significant more rabbits had sterile CSF at 5 and 10 hours, when treated with NZ2114 as compared to therapy with ceftriaxone (5/9 vs. 0/7 and 6/8 vs. 1/7, respectively, Fisher Exact test, $P<0.05$).

Conclusions: Due to its excellent CSF penetration and potent CSF bactericidal activity, the plectasin variant NZ2114 could be a new promising treatment option of Gram-positive CNS infections, including penicillin-resistant pneumococcal meningitis.

F1-3967

XF-73, A New Antimicrobial Drug With Rapid Membrane Activity

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Background: XF-73 is the lead compound in an entirely new class of anti-microbial agents, developed to address the growing and unmet need for prophylaxis and treatment of drug resistant bacteria such as methicillin-resistant *Staphylococcus aureus*. It has been previously demonstrated that the rapid bactericidal activity of XF-73 is not associated with significant cellular lysis. We report here further results on the mechanism of action of XF-73.

Methods: The integrity of the *S. aureus* cell membrane following exposure to XF-73 and control agents was determined using a range of techniques. Membrane potential was measured using a DiSC₃(5) fluorometric assay and leakage of intracellular potassium and magnesium was determined by atomic absorption spectroscopy. The sequence of biochemical events detected by these assays was compared with the killing kinetics displayed by XF-73 under identical conditions.

Results: Rapid reduction of membrane potential (>90%) and significant loss of intracellular cations occurred within 6 minutes of exposing *S. aureus* to 4x MIC concentrations of XF-73. This coincided with a 4 log drop in viable cell numbers. For daptomycin at 4x MIC, there was < 10% reduction in membrane potential, <10% leakage of potassium and <1 log drop in viable cells over the same time period.

Conclusions: The bactericidal anti-staphylococcal activity of XF-73 can be attributed to its rapid effects on the integrity of the cytoplasmic membrane. The >90% reduction of membrane potential, loss of intracellular cations and reduction of cell viability have been shown to occur simultaneously. Work is ongoing to further elucidate the mechanism of action and XF-73 is now in clinical development.

F1-3968

XF-73, A New Antimicrobial Drug, Active Against Biofilms and Slow Growing Cultures of *Staphylococcus aureus*

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Background: A key feature of biofilm formation is the bacteria's ability to resist antibiotic activity. The slower growth rate of bacteria in biofilms may be an important factor in increased resistance. We investigated the activity of XF-73, the lead compound in an entirely new class of antimicrobial agents, against biofilm and slow growing cultures of *Staphylococcus aureus*.

Methods: Minimum inhibitory concentrations (MICs) were determined for planktonic cultures by broth microdilution according to BSAC guidelines. Biofilm MICs (bMIC) and minimum biofilm eradication concentrations (MBECs) were determined using the Calgary biofilm device. The effect of XF-73 on the viability of cold culture cells was determined by growing *S. aureus* SH1000 to early exponential phase at 37°C and resuspending the cells in pre-chilled media where they were maintained in the presence and absence of XF-73 and controls. The effect of XF-73 on slow growing cells expressing the stringent response was determined by growing cultures to early log phase and the stringent response induced by the addition of the IRS inhibitor mupirocin. XF-73 and controls were then added and samples recovered for viable cell determinations.

Results: XF-73 had excellent antibiofilm activity with a bMIC of 1 µg/ml and a MBEC of 2 µg/ml against *S. aureus* SH1000 compared with bMICs of 4, 0.5 and 0.03 µg/ml and MBECs of >256, >256 and 128 µg/ml for ciprofloxacin, fusidic acid and rifampin respectively. Cold culture and stringent response cultures remained susceptible to XF-73 with a 5 log drop in viability observed within 1 hour compared with no loss of viability for cultures treated with fosfomycin.

Conclusions: The excellent activity of XF-73 against biofilms and slow growing *S. aureus* cultures demonstrates that its antibacterial activity is independent of the growth state of the bacteria and suggests additional utility for XF-73 in the treatment of biofilm associated infections.

F1-3969

A Novel XF Antibacterial, DPD-207, Against *Staphylococcus aureus* in a Murine Thigh Infection Model

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Background: As a novel class of antibacterials, XF drugs possess a unique mechanism of action, and the likelihood for the development of resistance to them is remote. The lead compound of the series has entered into clinical development as a topical anti-staphylococcal agent. We herein report the findings of *in vivo* studies assessing the systemic administration of DPD-207, a new member of the XF drug family.

Methods: CD-1 mice were immunocompromised and infected with 10^7 cfu/ml *Staphylococcus aureus* (SA) per thigh. DPD-207 was administered SC, IV or PO in dosages ranging from 0.25 to 40 mg/kg/day. Bacterial density in thigh tissue was assessed at 2-hour intervals from 0 to 8 hours and again at 24 hours in each treatment and control group. A one-way ANOVA was performed between groups at each time of sample collection with significance set at $P < 0.05$.

Results: Statistical evaluations demonstrated significant antibacterial effects over time for both IV and PO doses versus the control groups. IV administration of DPD-207 led to a significant ($P < 0.05$) anti-SA effect, although mortality was observed at doses ≥ 10 mg/kg. DPD-207 also demonstrated significant ($P < 0.05$) anti-SA activity when given via the oral route, with no apparent acute toxicity at doses up to 40 mg/kg. No significant anti-staphylococcal activity or any apparent acute toxicity was observed with the subcutaneous administration of DPD-207.

Conclusions: These results are the first *in vivo* studies of DPD-207 and show anti-SA activity of this XF drug after either IV or oral administration. While mortality was seen with the higher IV dosing regimens, none was observed after high oral doses. Further studies with DPD-207 are warranted, using formulations designed to enhance its activity profile. As the likelihood of resistance developing is remote, the XF compounds have the potential to be developed into anti-bacterial agents with a long lifetime of clinical utility.

F1-3970

XF-73: A New Antimicrobial - Comparison with Daptomycin for the Development of Mutational Resistance in MRSA USA300 in a 55-Passage Study

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Background: XF-73, the lead compound in a new class of bactericidal antimicrobial agents, has previously been shown to be potent (MIC₅₀ and MBC₅₀ 1 mg/L) against a range of *Staphylococcus aureus* strains including healthcare-associated methicillin-resistant *S. aureus* and community-associated methicillin-resistant *S. aureus* (CA-MRSA). The aim of this study was to compare the propensity of XF-73 and daptomycin to cause mutational resistance in USA300, the predominant CA-MRSA strain in the USA.

Methods: MICs were determined for XF-73 and daptomycin using a macrodilution broth method. USA300-0114 was passaged 55X at 0.5X MIC (determined from the previous passage) to investigate if there was an increase in the MIC, suggestive of the development of mutational resistance. Daptomycin MICs were determined in broth supplemented with calcium to a final concentration of 50mg/L.

Results: The initial MICs for XF-73 and daptomycin were 0.25 and 0.5 mg/L, respectively. A stepwise doubling in the MIC of daptomycin was observed during the course of the study, the MIC doubling being followed by a period of constant MIC before the next increase, with the MIC reaching 32 mg/L after 55 passages. A significant (³⁸-fold) increase in the MIC was observed after only 5 passages. In comparison, the MIC of XF-73 did not increase significantly (<8-fold increase), even after 55 passages (final MIC 0.5mg/L).

Conclusions: The emergence of stable resistance to daptomycin during passage studies has been reported previously. In this study, daptomycin resistance was found to emerge rapidly in USA300 while in contrast, no mutational resistance emerged for XF-73. Thus, XF-73 may have a significant advantage over daptomycin for clinical application.

F2-2070

Structural Guided Design of Pyrimidine Antibacterials Targeting Methionyl-tRNA Synthase (MetRS)

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Background: Enzyme-inhibitor co-crystal structures are of great utility in the discovery of new classes of antibacterial agents. Although structures of compounds bound to Gram-negative MetRS are known, there is significant sequence diversity between the Gram-negative and Gram-positive enzymes. Consequently, the structures of compounds bound to a Gram-positive MetRS ortholog are critical in the design of potent inhibitors with Gram-positive spectrum.

Methods: Multiple constructs of four Gram-positive orthologs were screened for crystallization using the Fluidigm Topaz instrument. The best construct, a truncated variant of the *S. aureus* enzyme, yielded crystals that allowed for 1.8Å structure determination by molecular replacement. Once enabled, subsequent iterations of structure-based design were conducted.

Results: Analysis of the structures of inhibitors bound to *S. aureus* MetRS identified several key interactions that provide enzymatic potency, including binding to Asp-51, and interactions with two distinct hydrophobic pockets. Initial structures also revealed an adjacent pocket, formed by the interface of two sub-domains of the enzyme, representing opportunities for additional interactions. Through several rounds of synthesis, testing, crystallography and design, we advanced a 100 micromolar MetRS virtual screening hit with no antibacterial activity to a series of pyrimidines with highly potent enzymatic (IC_{50} 1 nM) and antibacterial activity (MIC 1 μ g/mL). Further structure-based optimization provided compounds with increased solubility and no increase in MIC values in the presence of serum. In addition, we were able to rationally design compounds with novel interactions in the “interface cleft”, including a hydrogen bond with Tyr-235.

Conclusions: Iterative cycles of structure based drug design yielded compounds with both improved potency and drug properties including reduced serum binding. Continued use of structure-guided optimization provides a method to improve *in vivo* properties.