



Neue Gyrasehemmer

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Code	Name/Substanz	Gruppe	Wirksam gegen	Abstract
ACH-702	Isothiazolchinolon	Chinolon	Gram(+) Bakterien <i>Neisseria</i> spp. <i>Haemophilus</i> spp.	F1-2021 ; F1-2022 ; F1-2023 ; F1-2024 ; F1-2054
AM-3005	Mutilin-Chinolon	Chinolon	MRSA	F1-2030 ; F1-2031 ; F1-2032
--	Finafloxacin	Chinolon	Gn+GP Bakterien; <i>Helicobacter pylori</i>	F1-2036 ; F1-2037 ; F1-2039 ; F1-2040 ; F1-2041 ; F1-2042 ; F1-2043 ; F1-2044 ; F1-2045 ; F1-2046 ; F1-2047 ; F1-2048 ; F1-2049
DW-224a	Zabafloxacin	Chinolon	<i>Str. pneumoniae</i>	A-046 ; A-047 ; A-1895
JNJ-Q2	Fluorochinolon	Chinolon	MDR <i>Streptococcus pneumoniae</i>	F1-2033 ; F1-2035 ; F1-2052
TG873870	Nemofloxacin	Chinolon	MRSA; <i>Streptococcus</i> spp.; <i>Helicobacter</i>	B-056 ; B-1005 ; C1-189 ; C1-1957 ; C1-1971 ; C2-254 ; C2-3931 ; F1-2055 ; F1-2056 ; F1-2057 ; L-678
NXL 101	--	Chinolon	Staphylokokken; MRSA	F1-2053
TMC207	Diarylchinolon (R207910)	Chinolon	Mycobakterien	B-877

A-046

Efficacy of Dose-Ranging and Front-Loading Zabofloxacin (Zabo) for *Streptococcus pneumoniae* (Spn) in an *In Vitro* Pharmacodynamic (PD) Model

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Background: Zabo (DW-224a) is a new fluoroquinolone antibiotic (Abx) with potent *in vitro* activity against Spn. Seven-day dose-ranging studies were conducted in an *in vitro* PD model for a wild-type Spn (Spn-WT, Zabo MIC: 0.015 mg/L) and an isogenic strain that overexpresses efflux pumps (Spn-EP, Zabo MIC: 0.06 mg/L) to identify Zabo regimens that would optimize kill of the Abx-susceptible population and prevent emergence of resistance. For Spn-EP the effect of front-loading of Zabo on bacterial killing was also studied.

Methods: Zabo was given to PD-systems simulating the mean non-protein bound (29% free) human serum conc.-time profiles ($t_{1/2}$ 6 h) for dosages of 0 to 600 mg of Zabo given once daily (QD). The effect of 2 front-loading regimens of Zabo against Spn-EP was also studied (front loading regimen #1: 600 mg x 1 dose then 300 mg QD x 3 doses; regimen #2: 500 mg QD x 3 doses). Bacterial samples taken from each PD system were quantitatively cultured each day to define the effect of each regimen on the total Spn population and on resistance selection.

Results: For Spn-WT, Zabo 50 mg QD failed due to resistance selection. Zabo at ≥ 100 mg QD decreased the Spn-WT density from 10^6 CFU/ml to non-detectable levels (≤ 50 CFU/ml) within 24 h of therapy without resistance selection. For Spn-EP, Zabo 300 mg QD failed in trial #1 due to resistance selection but in trial #2, this simulated regimen rapidly reduced the bacterial density to non-detectable levels by day 1 of therapy and prevented resistance amplification. Zabo 400 mg QD and both front-loading regimens rapidly sterilized the PD systems that were inoculated with Spn-EP in both trials.

Conclusions: Zabo at a simulated dose of 100 mg QD rapidly killed Spn-WT and prevented emergence of resistance. For Spn-EP, Zabo at 400 mg QD and both front-loading regimens sterilized the PD systems. These data will be useful to select Zabo dosage regimens for Phase 2/3 studies.

A-047

Evaluation of the Inhibition Potential of Zabofloxacin (DW-224a) on CYP450 in Human Liver Microsomes

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Background: Zabofloxacin (ZAB) is a novel fluoroquinolone with potent *in vitro* activity against *S. pneumoniae*. This study was conducted to evaluate the inhibition potential of ZAB on CYP450 in human liver microsomes (HLM). **Methods:** P450 inhibition assays were performed in 96-well plates at 37°C. ZAB was diluted from a 10 mM H₂O stock and incubated in duplicate at eight final concentrations from 0.016 to 50µM in half-log steps. The assay was standardized for both phosphate buffer (75 mM, pH 7.4) and the NADPH regenerating system (MgCl₂, 3.3 mM; G6P, 3.3 mM; G6PD, 1 U/MI; NADP⁺, 1.3 mM); protein concentrations and incubation times were isoform-specific. Positive control inhibitors were diluted from 10 mM DMSO stocks and assayed as the test article (0.5% DMSO final incubation). Assays (200 µL) were mixed with HLM, diluted ZAB, and substrate before initiating the reaction with regenerating system mixture. Samples were stopped with acetonitrile, centrifuged and analyzed using LC-MS/MS. Formation of probe metabolites was measured by monitoring SRM transition.

Results: The IC₅₀ of ZAB was found to be >50 µM for all isoforms tested. **(siehe nächste Folie)** **Conclusions:** ZAB was shown not to inhibit the 7 major HLM of the CYP450 at concentrations up to 50 µM.

A-047 (Forts.)

Table 1. Summary ZAB IC₅₀ data

Sponsor ID	IC ₅₀ (µM)						
	1A2	2B6	2C8	2C9	2C19	2D6	3A4†
Zabofloxacin	>50	>50	>50	>50	>50	>50	>50

Table 2. Summary positive control IC₅₀ data

Positive Control ID	IC ₅₀ (µM)						
	1A2	2B6	2C8	2C9	2C19	2D6	3A4†
Furafylline	2.0	-	-	-	-	-	-
ThioTEPA	-	2.4	-	-	-	-	-
Montelukast	-	-	0.023	-	-	-	-
Sulphaphenazole	-	-	-	0.064	-	-	-
Benzylrivanol	-	-	-	-	0.22	-	-
Quinidine	-	-	-	-	-	0.031	-
Ketoconazole	-	-	-	-	-	-	0.012

†determined using midazolam

A-1895

Optimal Sampling Strategies (OSS) to Estimate the Area Under the Concentration-Time Curve at Steady-State (AUC_{0-24}) for Zabofloxacin (ZAB)

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Background: ZAB is a novel fluoroquinolone with potent *in vitro* activity against *S. pneumoniae*. Using Phase 1 PK data, OSS were developed to estimate ZAB AUC_{0-24} for a Phase 2 study of patients with community-acquired pneumonia.

Methods: PK data were obtained from a multiple-dose Phase 1 study. 18 male subjects were enrolled into 1 of 3 cohorts (200, 400 or 800 mg ZAB QD x 7), each with n=6. Data were fit by candidate PK models using non-linear regression (ADAPT 5) & were weighted by the estimated observation variance. Model discrimination was carried out using Akaike's Information Criterion. Multiple step-wise linear regression was used to determine concentration sampling times most predictive of AUC_{0-24} . For ZAB 300 mg, the fitted oral clearance was used to calculate AUC_{0-24} , the dependent variable; the observed PK data represented the candidate independent variables. Precision was computed as the % of the variability in AUC_{0-24} explained by the subset of plasma concentration-time points in the OSS.

Results: The best fit to the PK data was obtained with a 2 compartment model with an absorptive delay & 1st order absorption, inter-compartmental distribution & elimination. OSS ranging from 2-7 samples/patient & the associated precision are shown below.

Conclusion: ZAB PK was best described by a 2 compartment model with absorptive delay & 1st order absorption, inter-compartmental distribution & elimination. OSS to estimate ZAB AUC_{0-24} with 2-7 samples/patient show high precision but the best efficiency is seen with 4-5 samples/patient.

OS S	Recommended sampling times after administration of ZAB 300 mg (hours post-dose)								Precision (%)
2	2	8	-	-	-	-	-	-	95.3
3	2	6	8	-	-	-	-	-	96.9
4	0.75	2	6	8	-	-	-	-	98.3
5	0.75	1.5	3	6	8	-	-	-	99.5
6	0.75	1.5	3	6	8	12	-	-	99.7
7	0.75	1.5	3	4	6	8	12	-	99.9

B-056

In Vivo Efficacy of Nemonoxacin in a Mouse Pulmonary Infection Model

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Background: Nemonoxacin is a novel non-fluorinated quinolone with potent broad-spectrum activities against pathogens including multi-drug resistant *Streptococcus pneumoniae*. The purpose of this study was to compare the *in vivo* efficacy of nemonoxacin and moxifloxacin in a mouse pulmonary model of infection by *S. pneumoniae*.

Methods: Anesthetized mice were challenged intranasally with a lethal number of *S. pneumoniae*. Nemonoxacin and moxifloxacin was administered subcutaneously at 12, 18, and 24 h post-challenge at 50, 25, 12.5, or 6.25 mg/kg/total dose (9 or 11 per group). The number of viable bacteria in blood and lung was assessed at 28 h post-challenge. Lung was also evaluated histopathologically. Auxiliary animals were monitored for 7 days for survival.

Results: Nemonoxacin reduced viable bacteria counts in blood and lung when compared to vehicle-treated controls. A dose response was seen as nemonoxacin dosage increased. Nemonoxacin and moxifloxacin reduced viable bacterial counts by 5.6 - 6.6 and 2.9 - 6.6 logs in blood and 1.2 - 5.1 and 0.2 - 2.2 logs in lung tissue, respectively. Histopathologic evaluation of lung from nemonoxacin-treat mice showed minimal to mild alveolar/interstitial inflammation; no distinct differences were found when compared to moxifloxacin-treated mice. Nemonoxacin protected 100% of mice from death at all doses and no adverse clinical symptoms were noted in the 7 day observation period. In contrast, the survival rates for moxifloxacin were 0%, 17%, 50% and 100% at 6.25, 12.5, 25 and 50 mg/kg, respectively. Nemonoxacin was more effective in reducing the number of bacteria and protecting mice from mortality than moxifloxacin at the same doses.

Conclusions: Nemonoxacin demonstrated efficacy in a mouse pulmonary model infection by *S. pneumoniae*. These results suggest nemonoxacin could potentially be efficacy in humans and support further development of nemonoxacin in clinical studies.

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-1005

In Vivo Efficacy of Nemonoxacin in a Mouse Protection Model

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Background: This study compared the *in vivo* efficacy of nemonoxacin (Nemo) with those of 6 fluoroquinolones (FQ) against acute murine systemic infections by *S. aureus* (SA), *S. pneumoniae* (SP), *E. coli* (EC), ciprofloxacin-resistant SP (CR-SP), CR-methicillin-resistant SA (CR-MRSA).

Methods: Mice were challenged intraperitoneally with a single bacterial pathogen at lethal dose. Nemo and 6 FQs [including ciprofloxacin (Cipro), moxifloxacin (Moxi), levofloxacin (Levo), gatifloxacin (Gati), gemifloxacin (Gemi), and garenoxacin (Gare)] were administered subcutaneously (SC) or orally (PO) at 1 and 4 hr post-challenge to determine PD₅₀ (i.e., the drug dose required to protect 50% of mice from death) of each agent.

Results: PD₅₀ (mg/kg/dose): see table.

The PD50 values for Nemo SC and PO were: a) for SP, SA and CR-SP, lower than Cipro, Moxi, Levo, and Gati, and similar to Gemi and Gare; b) for EC, similar to or higher than all FQs; and c) for CR-MRSA, at least 2-fold lower than all FQs.

Conclusions: Nemonoxacin was comparable or more efficacious than currently marketed FQs tested in protecting mice from infections of gram-positive, gram-negative and drug-resistant bacteria.

Pathogen		Nemo	Cipro	Moxi	Levo	Gati	Gemi	Gare
SP	SC	2.2	22.3	8.4	15.2	5.8	2.3	4.0
	PO	4.8	48.3	7.2	25.0	11.0	8.6	4.8
SA	SC	1.1	13.9	3.0	9.6	2.5	1.1	0.4
	PO	2.1	9.3	2.7	6.6	3.5	1.0	0.8
EC	SC	0.6	0.1	0.4	0.1	0.3	0.2	0.6
	PO	3.7	0.5	1.2	0.6	0.7	2.9	1.8
CR-SP	SC	10.8	314.5	33.7	76.4	51.5	9.6	21.4
	PO	32.5	> 400	46.0	100.0	46.7	72.5	16.4
CR-MRSA	SC	99.5	412.9	491.0	414.2	199.0	432.3	246.9
	PO	105.0	> 400	276.5	> 400	209.9	> 400	189.6

C1-189

Comparative Antistaphylococcal Activity of Nemonoxacin, a Novel Broad-Spectrum Quinolone

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Background: Most MRSA are R to available quinolones. Nemonoxacin is a new non-fluorinated quinolone with expanded antistaph. and antipneum. activity. We tested activity of nemonoxacin, vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, quinupristin/dalfopristin, ciprofloxacin, levofloxacin, moxifloxacin against 88 MRSA.

Methods: Strains were 26 MRSA (quinolone S), 2 hetero vanco intermediate (hVISA), 24 vanco-intermediate (VISA), 5 vanco-resistant (VRSA), 31 quinolone R vanco-S MRSA with defined QRDR mutations. Sequencing of QRDR (*gyrA*, *gyrB*, *grlA*, and *grlB*), and efflux testing by reserpine, were done. Agar dil. MICs (CLSI), were used with added Ca²⁺ for dapto, 24 h incub. for vanco, fresh tige powder

Results: MICs (µg/ml) (**siehe nächste Folie**) Nemo had excellent activity against all strains including vanco non-S and quinolone R MRSA, with improved activity (MICs 0.06-4 µg/ml) compared to cipro (0.5->128 µg/ml), levo (0.5->32 µg/ml), moxi (0.06-8.0 µg/ml). Among 31 MRSA quinolone R strains 5 patterns of QRDR mutations [GyrA (S84L), GrlA (S80F/Y), GrlB (L413S, E422K/N, D432N, E471K); GyrA (S84L), GrlA (S80F/Y), GyrB (R404L); GyrA (S84L), GrlA (S80F/Y); GyrA (S84L), GrlA (S80F/Y, E84V), GrlB (E422D) and GyrA (S84L), GrlA (S80F/Y, E84V/K/G or S108N)] occurred. No nemo-associated efflux was found. Among genotypes known to be associated with R development, nemo had lowest MIC range compared to cipro (av. 64 x MICs increase), levo (av. 16 x MICs increase), moxi (av. 2-4 x MICs increase).

Conclusions: Nemo was potent against all MRSA tested irrespective of phenotype and had low MICs compared to those of other quinolones tested in strains with QRDR mutations causing quinolone R.

C1-189 (Forts.)

Drug	MRSA (26)			hVISA(2) + VISA (24) + VRSA (5)			Quinolone-res. MRSA (31)		
	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90
Nemonox	0.016-0.06	0.03	0.06	0.06-2	1	2	0.5-4.0	1	2
Ciproflox	0.25-2	1	1	0.5->128	64	>128	16->128	64	>128
Levoflox	0.25-1	0.25	0.5	0.5->32	16	32	4->32	16	32
Moxiflox	0.03-0.25	0.06	0.06	0.06-8	4	8	1-8	4	8
Vanco	0.5-1	1	1	1->32	4	32	0.5-2	1	1
Teico	0.5-1	0.5	1	1-32	8	16	0.25-2	0.5	1
Dapto	0.5-1	1	1	0.12-4	1	2	0.12-1	0.25	0.25
Linezolid	2-4	4	4	0.5-2	1	1	0.25-1	1	1
Tige	0.12-0.5	0.25	0.5	0.06-0.5	0.25	0.5	0.12-0.5	0.12	0.25
Quinu/dalfo	0.25-1	0.5	0.5	0.12-0.5	0.5	0.5	0.25-0.5	0.5	0.5

C1-1957

Activity of Nemonoxacin, an Investigational C8-methoxy Non-fluorinated Quinolone, Against Gram-Negative Bacilli Obtained From Canadian Hospitals: CANWARD 2007

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Background: Nemonoxacin (Nemo) is a novel C8-methoxy non-fluorinated quinolone. The purpose of this study was to assess the activity of Nemo against gram-negative bacilli obtained from Canadian hospitals as part of the CANWARD 2007 study.

Methods: From Jan-Dec 2007, 12 sentinel hospitals across Canada submitted isolates from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units. 7881 isolates were collected including 3,306 gram-negative bacilli. Susceptibility testing was performed using CLSI broth microdilution.

Results: MIC₅₀ and MIC₉₀ values for Nemo and levofloxacin (Levo) are shown below.

Conclusion: Nemonoxacin demonstrated similar activity to levofloxacin against gram-negative bacilli. Isolates demonstrating reduced susceptibility or resistance to levofloxacin demonstrated reduced susceptibility or resistance to nemonoxacin.

Organism (# isolates)	Nemo MIC ₅₀ /MIC ₉₀	Levo MIC ₅₀ /MIC ₉₀
<i>E. coli</i> (1701)	0.12 / >4	≤0.06 / 16
<i>P. aeruginosa</i> (633)	1 / >4	2 / 16
<i>K. pneumoniae</i> (455)	0.25 / 2	≤ 0.06 / 1
<i>E. cloacae</i> (166)	0.12 / 0.5	≤ 0.06 / 1
<i>P. mirabilis</i> (118)	0.5 / >4	0.12 / 4
<i>S. maltophilia</i> (106)	4 / >4	2 / 8
<i>K. oxytoca</i> (100)	0.25 / 0.5	≤0.06 / 0.12
<i>A. baumannii</i> (27)	0.25 / 0.5	0.25 / 0.5

C1-1971

In Vitro Resistance Development to Nemonoxacin for *Streptococcus pneumoniae*

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Background: This study assessed the potential for clinical isolates of *S. pneumoniae* (SP) to develop resistance to nemonoxacin (Nemo) and characterized gyrase and topoisomerase IV gene mutations, target genes of quinolone, in the selected resistant isolates.

Methods: Three unrelated clinical isolates of SP were exposed to Nemo in agar at ascending concentrations from 0.5 to 8-fold the drug minimum inhibitory concentration (MIC) for 3 selection cycles. As control, One clinical isolate was exposed to ciprofloxacin (Cipro) as a control. The MICs of Nemo, Cipro, moxifloxacin (Moxi), gatifloxacin (Gati), and levofloxacin (Levo) for the potential mutant colonies were measured after each cycle; selected mutations in the quinolone resistance-determining regions (QRDR) of *gyrA*, *gyrB*, *parC*, and *parE* were sequenced.

Results: The MICs of Nemo for the 3 Nemo-selection isolates increased 2-8-fold over 3 cycles of selection (initial MICs: 0.03-0.06 µg/ml; final MICs: 0.06-0.5 µg/ml), obtaining no highly resistant isolates. The comparative final MICs were 0.12-1 µg/ml for Moxi, 0.25-2 µg/ml for Gati, and 1-4 µg/ml for Levo and Cipro. In contrast, the MIC of Cipro for the Cipro-selection isolate increased 64-128-fold over 3 selection cycles (initial MIC: 0.5 µg/ml; final MICs: 32-64 µg/ml), yielding highly resistant isolates. The comparative final MICs were 0.5-1 µg/ml for Nemo, 4 µg/ml for Moxi, 4-8 µg/ml for Gati, and 16 µg/ml for Levo. QRDR mutations identified after exposure to Nemo included Ser82-Tyr in *gyrA*, Ser494-Thr in *gyrB*, and Pro454-Ser in *parE*. The mutations in the Cipro-resistant isolates were Ser79-Tyr in *parC* and Ser81-Tyr in *gyrA*, consistent with the previous publications.

Conclusions: Nemonoxacin appears to develop a unique resistance profile (e.g. no *parC* mutation) and be less prone to develop resistant by nemonoxacin compared to other fluoroquinolones.

C2-254

Multi-Drug Resistant *Streptococcus pneumoniae* Containing *erm(B)* and *mef(A)*

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Background: Aims: 1) To investigate the presence of the multi-drug resistant (MDR) *S. pneumoniae* (SP) strain, clonal complex (CC) 271 and serotype 19A in Europe, previously found extensively in North America, South Africa and the Far East. 2) To characterize the *mef* gene in SP isolates originating from the US and Europe, containing both *erm(B)* and *mef*.

Methods: Clinical isolates of SP from antimicrobial surveillance studies in the US and Europe were used. Isolates included were Western European SP with *erm(B)* and *mef* (n = 44), Eastern European SP with *erm(B)* and *mef* (n = 56) and US SP with *erm(B)* and *mef*, not serotype 19A or 19F (n = 12).

Characterization of the *mef* gene was carried out using a novel TaqMan™ assay. Determination of the presence of *erm(B)* and *mef* was performed using a multiplex PCR method. Serotyping was performed using the Neufeld's Quellung reaction. MLST was performed using previously published protocols.

Results: MLST results showed the presence of 5 clonal complexes (CC15, CC81, CC87, CC271 and CC242) and 2 individual sequence types ST143 and ST473. Serotyping identified 6 different serotypes 14, 19A, 19F, 23F and 6A. Five isolates were non-typeable.

Eleven (11) of the 100 European isolates tested were serotype 19A and CC271. The *mef* gene in isolates containing both *erm(B)* and *mef* were all characterized as *mef(A)* subclass *mef(E)*.

Conclusions: The MDR SP CC271, serotype 19A extensively found in North America, South Africa and the Far East has for the first time been identified in Europe. This clone is resistant to the macrolides, penicillin, tetracycline and cotrimoxazole. Particular significance is given to the fact that the pneumococcal vaccine PCV7 is not effective against this strain. The *mef* subclass *mef(A)* was not associated with *erm(B)* in these isolates containing the double macrolide resistance genes *erm(B)* and *mef*.

C2-3931

In Vitro Activity of Nemonoxacin Against *Helicobacter pylori*

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Background: Nemonoxacin (TaiGen Biotechnology), a novel non-fluorinated quinolone, has potent and broad-spectrum *in vitro* activity against Gram-positive, Gram-negative, and atypical pathogens. This study aimed to evaluate the antibacterial activity of nemonoxacin along with four other fluoroquinolones against clinical isolates of *Helicobacter pylori*.

Methods: Minimum inhibitory concentrations (MICs) of nemonoxacin, ciprofloxacin, levofloxacin, moxifloxacin, and gemifloxacin for 200 isolates of *H. pylori* (2000-2007) were determined using the agar dilution methods recommended by the Clinical and Laboratory Standards Institute (CLSI-M100-S18).

Results: Rate of presumptive resistance to amoxicillin (MICs ³⁰0.5 µg/ml), clarithromycin (MICs ³¹ µg/ml, CLSI), metronidazole (MET) (MICs ³⁸ µg/ml), ciprofloxacin (MICs ³¹ µg/ml), and levofloxacin (MICs ³¹ µg/ml) was 2%, 6%, 29%, 2%, and 2%, respectively. The MIC range (MIC₉₀) of the isolates to ciprofloxacin, levofloxacin, moxifloxacin, gemifloxacin, and nemonoxacin was 0.12-2 µg/ml (0.5 µg/ml), 0.12-1 (0.5), 0.12-4 (0.5), £0.03-0.5 (0.12), and 0.06-1 (0.25), respectively.

Conclusions: Nemonoxacin had an excellent *in vitro* activity against *H. pylori* isolates, including clarithromycin-, metronidazole-, and levofloxacin-resistant isolates. The MIC₅₀/MIC₉₀ values of nemonoxacin for the isolates were lower than those of ciprofloxacin, levofloxacin, and moxifloxacin, and comparable to those of gemifloxacin.

F1-2021

In Vitro and In Vivo Antibacterial Activities of ACH-702 against Gram-Positive Pathogens

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Background: ACH-702 is an isothiazoloquinolone that exhibits excellent activity against most gram-positive pathogens.

Methods: The antibacterial activity of ACH-702 was assessed against gram-positive and gram-negative clinical isolates by several standard *in vitro* and *in vivo* methods.

Results: MIC assays revealed that ACH-702 displayed broad-spectrum activity, especially against gram-positive bacteria, with MICs for most strains $\leq 2 \mu\text{g/ml}$. These included antibiotic-resistant isolates such as methicillin-resistant *S. aureus* (MRSA), vancomycin non-susceptible staphylococci, and quinolone-resistant strains. For gram-negative bacteria, reduced activity was observed against some *Enterobacteriaceae* strains, but there was exceptional potency against *H. influenzae*, *M. catarrhalis*, and *Neisseria* sp. with MICs $\leq 0.06 \mu\text{g/ml}$ for all strains tested. Good antibacterial activity was also seen against several anaerobes as well as *L. pneumophila* and *M. pneumoniae*. Excellent bactericidal effects were observed against staphylococci in time-kill assays with a ≥ 3 -log drop in CFU/ml after 4 hours of exposure. Postantibiotic effects (PAEs) of 1.2-4.6 h were seen in staphylococci including clinical isolates after treatment with $10 \times$ MIC ACH-702, similar in most cases to those observed for moxifloxacin at $10 \times$ MIC. In vivo efficacy was demonstrated against *S. aureus* in murine sepsis, lung, and thigh infection models. PD50 values were $\leq 1 \text{ mg/kg}$ in sepsis infections while, in thigh infections, decreases in CFU/thigh were equal to or greater than those observed after vancomycin treatment.

Conclusions: ACH-702 displays potent, bactericidal activity especially against gram-positive pathogens and demonstrates efficacy in animal infection models as well.

F1-2022

Dual Targeting of Gyrase and Topoisomerase IV by ACH-702 and Mutation Analysis in *Staphylococcus aureus*

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Background: ACH-702 is an isothiazoloquinolone (ITQ) with potent antibacterial activity against *Staphylococcus aureus*. Selection of resistant mutants and the target genes involved were investigated.

Methods: Standard *in vitro* methods were used to analyze resistance and antibacterial mechanisms of action. These included isolation of mutants on solid medium containing compound and biochemical enzyme assays to calculate enzyme inhibition.

Results: First-step mutants of *S. aureus* ATCC 29213 (parent MIC = 0.004 µg/ml) were present exclusively in the DNA gyrase gene, *gyrA*, but not in the topoisomerase IV gene, *griA*, with low frequencies of resistance (10^{-9} - 10^{-10}). Low MIC values, £0.016 µg/ml, against mutants with single mutations in either *gyrA* or *griA* suggested that ITQs possessed significant inhibitory activities against both target enzymes. This dual target inhibition was supported by low µM IC₅₀ values against both topoisomerase IV (~1 µM), measured in a decatenation activity assay, and DNA gyrase (~1-10 µM), measured in a supercoiling activity assay. Retention of good MICs (£1 µg/ml) against staphylococcal *gyrA-griA* double mutants, as well as low frequencies of higher-level resistance (10^{-9} - 10^{-10}), indicated that ITQs remained active against both mutant enzymes. This was supported by demonstration of *in vitro* inhibition of both mutant enzymes with IC₅₀ values about 10-fold higher than against wild type enzyme. Macromolecular synthesis inhibition assays confirmed that ACH-702 inhibits DNA synthesis in *S. aureus*.

Conclusions: Isothiazoloquinolones display good antibacterial activities against both laboratory mutants and clinical MRSA strains with multiple mutations in topoisomerases. Low spontaneous resistance frequencies and an apparent dual-targeting mode of action with exceptional activity against DNA gyrase suggest the potential utility of ITQs in combating infections caused by *S. aureus*, including multi-drug resistant MRSA.

F1-2023

Bactericidal Activity of ACH-702 against Non-dividing Staphylococci

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Background: In many clinical bacterial infections, the causative pathogens are often found in environments that are not conducive for optimal growth. Non-dividing bacterial cells are killed less well by most currently used antibacterial agents. The isothiazoloquinolone, ACH-702, has shown rapid bactericidal activity against exponential phase bacterial cultures. The purpose of this work was to examine the bactericidal activity of ACH-702 against non-dividing staphylococci.

Methods: Bactericidal activity against both exponential- and stationary-phase staphylococci was determined in Mueller-Hinton (MH) broth. Exponential cells were growth arrested by nutrient depletion, antibiotic exposure, or environmental treatment (cold temperature) prior to addition of ACH-702. Cell viability was measured by dilution and plating onto MH agar plates.

Results: ACH-702 retained dose-dependent bactericidal activity against non-dividing staphylococci with a dose response observed up to 32[×] MIC. *S. aureus* ATCC 29213 stationary-phase cells showed a 3-log reduction in viability within 2 hours at 20[×] MIC and cells that were growth-arrested by protein synthesis inhibitors were similarly susceptible as well. This was in contrast to quinolones and other antibiotic comparators that showed little or no killing of non-dividing cells. Bactericidal activity was not observed against staphylococci whose growth was arrested by cold temperature. ACH-702 also demonstrated rapid bactericidal activity against staphylococcal biofilms in contrast to comparator compounds.

Conclusion: ACH-702 was bactericidal against non-dividing staphylococcal cells whose growth was stopped by either nutrient depletion or protein synthesis inhibition. Because few other antibiotics possess this property, further investigation of this activity is warranted.

F1-2024

Pharmacokinetic/Pharmacodynamic (PK/PD) Factors Influencing Emergence of Resistance to ACH-702 in Methicillin- and Quinolone-Resistant *Staphylococcus aureus* (MQRSA) in an In Vitro Model (IVM)

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Background: The isothiazoloquinolone ACH-702 displays rapid bactericidal activity against MQRSA in vitro and a low propensity to select resistant mutants. However, the relationship between ACH-702 PK/PD and the emergence of resistance in MQRSA is not known.

Methods: Log-phase cultures (8 log CFU/ml) of two ACH-702-susceptible (MIC 0.125 µg/ml) MQRSA strains, 8043 C96-7 (S80F *grlA*, S84L *gyrA*) and 8282 L96-22 (S80F/P144S *grlA*, E422D *grlB*, S84L *gyrA*), were exposed for 96 h in a hollow fiber IVM to an ACH-702 PK profile designed to achieve a target free-drug (*f*) AUC/MIC of 30, based on the proposed PK/PD breakpoint for quinolone efficacy against *S. aureus*. The ACH-702 *t*_{1/2} simulated was 6 h based on allometric scaling estimates of human clearance derived from animal studies, and the drug was given every 12 h. The development of resistance was evaluated by population analysis profiling, and the resistance phenotypes/genotypes of resistant subpopulations that emerged were characterized.

Results: When exposed to ACH-702 *f*AUC/MICs of 31.6 and 31.2 and C_{max}/MIC of 2.0 and 1.9, viable counts of 8043 C96-7 and 8282 L96-22 declined initially to 3 and 5 log CFU/ml at 72 and 36 h, respectively, and then increased. Subpopulations of both strains that were resistant to ACH-702 concentrations of ³ 0.25 µg/ml were selectively enriched and the ACH-702 MICs for both strains increased 4-fold by 96 h. The MICs of post exposure isolates were lowered 4-fold by reserpine. Resistant bacteria recovered following the 96-h exposure had no additional mutations in the quinolone resistance-determining regions of *grlA/B* or *gyrA*, and no new mutations in *gyrB*.

Conclusion: ACH-702 *f*AUC/MIC ratios of ~30 and C_{max}/MIC of ~2 are not sufficient to prevent the selection of low-level resistant variants present in susceptible MQRSA populations.

F1-2030

AM-3005: Synthesis and In Vitro Antibacterial Activity of Novel Mutilin-Quinolone Hybrid Antibacterial Agent

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Background: The continuous emergence of multiple drug-resistant Gram-positive bacteria led us to develop more effective antibacterial agents. The hybrid antibacterial agent incorporating multiple pharmacophores in one molecule, by addressing the active site of different targets, offer the possibility to overcome existing bacterial resistance and to avoid introducing new resistance. In the course of our quest to develop a potent antibacterial agent against multiple drug-resistant Gram-positive bacteria, we designed and synthesized novel mutilin-quinolone hybrids.

Methods: A series of mutilin-quinolone hybrids having the both of a mutilin and a 1-substituted-4-oxo-3-quinolinecarboxylic acid moieties was synthesized and in vitro antibacterial activity of these compounds were evaluated.

Results: Among the compounds evaluated, AM-3005 displayed extremely potent antibacterial activity (MICs, $\mu\text{g/mL}$) against *S. aureus* Smith (0.008), MRSA L39 (0.008), quinolone resistant clinical isolate *S. aureus* OITI MR1-1002 (0.016), *S. pneumoniae* Type III (0.031), PRSP PR44 (0.016), quinolone resistant *S. pneumoniae* No.55 (0.031), and vancomycin resistant *E. faecium* A2280 (0.016) compared with SB-264128 (0.125, 0.125, 0.125, 0.5, 0.5, 0.5, and 0.5) and ciprofloxacin (0.25, 0.125, 32, 0.5, 1, 64, and 128).

Conclusions: AM-3005 is the novel mutilin-quinolone hybrid antibacterial agent and a promising candidate for further evaluation.

F1-2031

AM-3005, a Novel Mutilin-Quinolone Hybrid Antibiotic with Potent Activity against MRSA

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Background: The continuous emergence of MRSA requires urgent development of more effective antibacterial agents. AM-3005 is a novel mutilin-quinolone hybrid antibiotic with potent activity against Gram-positive bacteria. The aim of this study was to evaluate the in vitro and in vivo activity of AM-3005 against MRSA.

Methods: MICs were determined by agar dilution method according to CLSI guidelines. The in vivo efficacy was evaluated in mouse systemic infection models caused by MSSA and MRSA.

Results: The activities of AM-3005 and other drugs against recent MRSA clinical isolates in Japan were as follows (see table). MIC₉₀ of AM-3005 against MRSA was 16- and over 8000-fold lower than that of SB-264128 (SB) and ciprofloxacin, the representatives of each pharmacophore, respectively. MIC of AM-3005 was 0.031 µg/mL or less against all the MRSA strains. AM-3005 was more active compared with other anti-MRSA drugs tested.

The ED₅₀s (mg/kg) of AM-3005, ciprofloxacin, and SB against the mouse systemic infection after i.v. dosing were 3.7 (MSSA) and 8.0 (MRSA), 2.1 and >50, and 1.6 and 6.4, respectively. In vivo efficacy of AM-3005 against MRSA infection model was almost the same as that against MSSA model.

Conclusions: AM-3005 is effective against the infections caused by MRSA. Therefore, it is promising compound for further evaluation.

Organism (n)	Drugs	MIC (µg/mL)			
		Range		50%	90%
MRSA (58)	AM-3005	0.008	– 0.031	0.016	0.016
	Ciprofloxacin	8	– >128	64	>128
	SB-264128 (Mutilin analogue)	0.125	– 0.25	0.25	0.25
	Quinupristin / Dalfopristin	0.25	– 0.5	0.25	0.5
	Linezolid	0.5	– 1	1	1
	Vancomycin	0.5	– 2	1	1
	Daptomycin	0.5	– 1	1	1
Oxacillin	64	– >128	>128	>128	

F1-2032

AM-3005 (AM), a Novel Mutilin-Quinolone Hybrid, Possesses Potent Inhibitory Activity against Protein Synthesis with a Type II Topoisomerase Inhibition

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Background: AM is a novel mutilin-quinolone hybrid with potent activity against Gram-positives. The aim of this study was to characterize the antibacterial profile of AM.

Methods: Inhibitory activities against Gyrase and Topoisomerase IV from *S. aureus* were determined by supercoiling and decatenation assay, respectively. In vitro transcription/translation (T/T) assay was performed with the *E. coli* S30 Extract System. Gene mutation was analyzed in the resistant mutants obtained in this study.

Results: The MIC of AM against *S. aureus* RN4220 was 16- and 128- fold lower than that of SB-264128 (SB, Mutilin analogue) and Ciprofloxacin (Cip), the representatives of each pharmacophore, respectively. AM potently inhibited protein synthesis with an IC_{50} of 0.35 μ M by T/T assay, and this inhibitory activity was almost equal to SB. Also, AM possessed inhibitory activities against Gyrase and Topoisomerase IV with IC_{50} of 123 and 357 μ M, respectively. However, these inhibitory activities were lower than those of Cip. According to macromolecule synthesis study, AM completely inhibited protein synthesis and decreased to 68 % of DNA synthesis and 37 % of RNA synthesis at 8 x MIC. Spontaneous AM-mutants possessed a mutation in the *rpmV* gene encoding the L22 ribosomal protein. AM-mutants showed lower susceptibility to SB, however, exhibited no cross-resistance to other protein synthesis inhibitors, such as oxazolidinone, macrolides, aminoglycosides, lincomycin, chloramphenicol, and tetracycline.

Conclusions: AM inhibits Type II topoisomerases as well as protein synthesis, which seems to be a primary target. These inhibitory activities against protein, DNA, and RNA synthesis might be contributed to higher antibacterial activity of AM compared with those of SB and Cip. Moreover, mode of action for protein synthesis of AM might be different from those of other protein synthesis inhibitors except SB.

Therefore, it is promising compound for further evaluation.

F1-2033

Broad Spectrum In Vitro Activity of JNJ-Q2, a New Fluoroquinolone

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Background: JNJ-Q2 is a new fluoroquinolone with broad-spectrum activity including MRSA and levofloxacin-resistant pneumococci. The in vitro activities of JNJ-Q2 were examined in comparison with other fluoroquinolone agents against a collection of other Gram-positive and Gram-negative isolates.

Methods: Broth microdilution MICs (CLSI methodology) were determined against *S. epidermidis*, *S. pyogenes*, *H. influenzae*, *E. faecalis* and Enterobacteriaceae isolates. In addition, 20 ciprofloxacin (CIP)-resistant *E. coli* isolates were tested for susceptibility.

Results: JNJ-Q2 displayed potent in vitro activity against the tested Gram-positive pathogens, with MIC₉₀ values of ≤ 0.015 $\mu\text{g/mL}$ against *S. pyogenes* as well as 0.015 and 0.25 $\mu\text{g/mL}$ against methicillin-susceptible and -resistant *S. epidermidis* (MSSE and MRSE), respectively. MIC ranges for the MSSE and MRSE isolates were 0.008 - 0.03 and ≤ 0.015 - 0.25 $\mu\text{g/mL}$, respectively. These activities were ≥ 8 -fold more potent than moxifloxacin (MOX). JNJ-Q2 displayed an MIC range of 0.03 - 1 $\mu\text{g/mL}$ and an MIC₉₀ value of 0.5 $\mu\text{g/mL}$ against *E. faecalis*. Against Gram-negative pathogens, JNJ-Q2 displayed MIC₉₀ values of 0.015 $\mu\text{g/mL}$ for *H. influenzae*, 0.12 $\mu\text{g/mL}$ for *E. cloacae* and 0.25 $\mu\text{g/mL}$ for *E. aerogenes*. Against *E. coli*, JNJ-Q2 displayed an MIC range of 0.015 - 0.5 $\mu\text{g/mL}$ and an MIC₉₀ of 0.25 $\mu\text{g/mL}$, equivalent to CIP. Against an independent set of CIP-resistant *E. coli*, JNJ-Q2 was 8-fold more potent than MOX. The JNJ-Q2 MIC range and MIC₉₀ for a collection of *K. pneumoniae* isolates was ≤ 0.015 - 1 $\mu\text{g/mL}$ and 0.25 $\mu\text{g/mL}$, respectively, values that were equivalent to those of CIP.

Conclusions: In vitro susceptibility testing demonstrated activity of JNJ-Q2 against a range of Gram-positive and Gram-negative pathogens, beyond MRSA and levofloxacin-resistant pneumococci. The activities of JNJ-Q2 against Gram-negative pathogens were, in general, comparable to moxifloxacin. The activities of JNJ-Q2 against Gram-positive pathogens were more potent than those of moxifloxacin.

F1-2035

In Vitro *Staphylococcus aureus* Activities and Resistance Selection in MRSA with the New Fluoroquinolone JNJ-Q2

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Background: JNJ-Q2 is a new broad-spectrum fluoroquinolone active against Gram-negative pathogens and multi-drug resistant *S. pneumoniae*. The activities and resistance development of JNJ-Q2 were examined against a set of MRSA isolates, including ciprofloxacin (CIP)-resistant and recent community isolates.

Methods: Broth microdilution MICs (CLSI methodology) were determined against 136 *S. aureus* isolates, including 110 MRSA isolates from hospital and community settings. Time-kill kinetics were determined according to CLSI methodology. Resistance development was characterized through the determination of resistance frequencies in single-step mutation studies. Resistance rates were determined through fluctuation tests. Multi-step serial passage studies were also performed, and the quinolone resistance determining regions of the genes encoding DNA gyrase and topoisomerase IV were amplified by PCR and sequenced.

Results: JNJ-Q2 displayed MIC₉₀ values of 0.015 and 0.5 µg/mL against MSSA and MRSA isolates, respectively. These activities were ≥8-fold more potent than moxifloxacin or linezolid. At 2X MIC, JNJ-Q2 was rapidly bactericidal against a CIP-R MRSA isolate. Resistance frequencies determined for five MRSA isolates for JNJ-Q2 at 2X MIC ranged from 4×10^{-9} to $<5 \times 10^{-10}$. Fluctuation tests revealed MRSA mutation rates to be much lower for JNJ-Q2 than for CIP. Resistance rates to CIP in MRSA occurred at 3×10^{-10} mutations per cell per generation, while for JNJ-Q2 mutation rates ranged from undetectable to 9×10^{-11} mutations per cell per generation. DNA sequence analysis in serial passage studies demonstrated that DNA gyrase is the primary target of JNJ-Q2 in *S. aureus*.

Conclusions: JNJ-Q2 demonstrated potent anti-staphylococcal activity in vitro, including against CIP-R isolates. JNJ-Q2 also demonstrated a low propensity for resistance development in MRSA isolates and lower resistance frequencies and resistance rates in MRSA in comparison with CIP.

F1-2036

New Fluoroquinolone Finafloxacin HCl (FIN): Route of Synthesis, Physicochemical Characteristics and Activity under Neutral and Acid Conditions

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Background: FIN, a novel fluoroquinolone (FQ), is a representative of a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH under which other FQs lose activity. FIN is therefore intended for therapeutic use against bacterial infections in acidic environments, e.g. *H. pylori* eradication, UTI.

Methods: I and II were synthesized and combined to III (FIN). Physicochemical characterization was performed by; NMR, X-ray, HPLC (solubility), titration (ionisation constants). MICs were determined using CLSI methodology for broth microdilution at different pH.

Results: I and II were synthesized in 7 steps at ~25% and ~30% yield, respectively. Coupling of I and II and subsequent crystallization into FIN resulted in ~55% yield.

Characterization of FIN included elucidation of the chemical and crystal structure, determination of solubility (mmol/mL; 12.5 (pH 7), 4.3 (pH 4.5)) and ionisation constants ($pK_{a1}=5.6$, $pK_{a2}=7.8$). FIN MICs ($\mu\text{g/mL}$) against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were 0.06 and 0.25 (pH 7.2) and 0.008 and 0.06 (pH 5.8), respectively.

Conclusions: FIN displays exceptional antibacterial activity at low pH, unlike other FQ, making it a prime candidate to treat infections in acidic environments, such as the gastrointestinal or urogenital tract.

F1-2037

Effect of pH on the In Vitro Activity of Finafloxacin against Gram-negative and Gram-positive Bacteria

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Background: Finafloxacin (FIN) is a novel 8-cyano-fluoroquinolone, that exhibits an *in vitro* spectrum of activity similar to that of ciprofloxacin (CIP). The present study was performed to study the effect of pH on the in vitro activity of FIN in comparison to CIP against selected strains of various aerobic Gram- and Gram+ bacterial species known to cause genito-urinary tract infections.

Methods: The susceptibilities of 100 clinical isolates to FIN and CIP were tested at pH 5, 6, 7.3, and 8. There were 22 *Escherichia coli* (ECO), 13 *Klebsiella pneumoniae* (KPN), 11 *Morganella morganii* (MOM), 10 *Proteus mirabilis* (PRM), 10 *Pseudomonas aeruginosa* (PSA), 12 *Staphylococcus aureus* (SAU), 11 *Staphylococcus saprophyticus* (SSA), and 11 *Streptococcus agalactiae* (SAG). Of these, 66 were sensitive and 34 exhibited reduced susceptibilities to CIP. MICs were determined using the CLSI broth microdilution method.

Results: Results are presented in Tables 1 and 2. (**siehe nächste Folie**)

Conclusions: Overall, FIN demonstrated superior activity to CIP under acidic conditions against isolates of all species including resistant strains. Furthermore, FIN showed comparable activity to CIP against Gram+ cocci at pH 7.3. Hence, FIN appears to be a promising new antimicrobial agent for the treatment of infections at acidic sites.

F1-2037 (Forts.)

Table 1: MIC-50/90s (mg/L) of FIN and CIP for Gram- bacteria

Species	FIN - pH 5/6/7.3/8		CIP - pH 5/6/7.3/8	
	MIC-50	MIC-90	MIC-50	MIC-90
ECO (n=22)	0.063/0.031/ 0.25/0.5	16/16/ ≥32/≥32	0.5/0.25/ 0.031/≤0.008	≥16/≥16/ ≥16/≥16
KPN (n=13)	0.5/0.5/ 2/2	8/8/ ≥32/≥32	≥16/8/ 2/0.5	≥16/≥16/ ≥16/8
MOM (n=11)	0.25/0.25/ 1/2	4/4/ 16/16	1/0.063/ ≤0.008/≤0.008	≥16/≥16/ 4/2
PRM (n=10)	0.125/0.25/ 1/1	4/4/ 16/16	1/0.125/ 0.016/0.016	≥16/8/ 2/1
PSA (n=10)	2/2/ 8/16	≥32/≥32/ ≥32/≥32	2/1/ 0.5/0.5	≥16/≥16/ ≥16/≥16

Table 2: MIC-50/90s (mg/L) of FIN and CIP for Gram+ bacteria

Species	FIN - pH 5/6/7.3/8		CIP - pH 5/6/7.3/8	
	MIC-50	MIC-90	MIC-50	MIC-90
SAU (n=12)	0.125/0.063/ 0.25/0.5	8/4/ 8/≥32	2/1/ 0.5/0.5	≥16/≥16/ ≥16/≥16
SSA (n=11)	0.063/0.125/ 0.5/1	0.125/0.125/ 0.5/1	0.5/0.5/ 0.25/0.5	1/0.5/ 0.5/0.5
SAG (n=11)	0.25/0.25/ 1/2	0.5/0.5/ 2/4	2/1/ 1/1	4/2/ 2/2

F1-2039

Comparisons of Methods for Finafloxacin MIC Testing at Acidic and Neutral pH

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Background: Finafloxacin (FIN) is a novel 8-cyano fluoroquinolone (FQ) that exhibits optimal activity at slightly acidic conditions (pH 5-6) where other FQs lose activity. FIN is intended for therapy of bacterial infections associated with an acidic environment such as *H. pylori* eradication and UTI. MIC testing of aerobic bacteria with FIN at acidic and neutral pH was studied by comparing Etest, CLSI agar (AD) and broth (BMD) dilution methods.

Methods: Etest, AD and BMD methods (MIC 0.002-32 µg/mL) were used to determine the MIC of FIN at pH 5.8 and pH 7.3. Challenge strains (39) used were: *E. coli* (8), *E. cloacae* (2), *A. anitratus* (1), *K. pneumoniae* (2), *P. vulgaris* (1), *P. rettgeri* (1), *P. stuartii* (1), *P. aeruginosa* (3), *S. marcescens* (2), *S. aureus* (13), *S. saprophyticus* (1), *S. haemolyticus* (3), *S. warnerii* (1). Quality control (QC) results for *E. coli* ATCC® 25922, *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 were generated and inoculum effects (100-fold) studied.

Results: Excellent inter-method MIC agreements were seen for results compared at the same pH. Species specific pH effects were seen and MIC values were 1-3 dilutions lower at acidic pH. QC results fell within specifications provided by the drug manufacturer. Inoculum effects on MIC values were minimal.

Conclusions: MIC testing of FIN with Etest, agar and broth dilution reference methods provides substantially equivalent results at both neutral and acidic pH, and demonstrates higher activity of FIN at slightly acidic pH. Etest with a wide concentration range (15 dilutions) comprise a useful MIC tool for drug development studies with FIN and for future studies with *H. pylori*.

Comparator	Regression analysis		% Agreement ± 1 dilution
	Equation	r	
AD vs. BMD, pH 5.8	$y = 0.94 x + 0.27$	0.99	100
AD vs. BMD, pH 7.3	$y = 0.97 x + 0.36$	0.98	100
Etest vs. AD, pH 5.8	$y = 1.11 x - 0.45$	0.97	87.5
Etest vs. AD, pH 7.3	$y = 0.99 x + 0.28$	0.99	100
Etest vs. BMD, pH 5.8	$y = 1.05 x - 0.27$	0.97	87.5
Etest vs. BMD, pH 7.3	$y = 0.97 x + 0.55$	0.98	97.9

F1-2040

MIC Testing of *Helicobacter pylori* Using Etest Finafloxacin and the Reference Agar Dilution Method

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Background: Finafloxacin (FIN) is a novel 8-cyano fluoroquinolone (FQ) that exhibits optimal activity at slightly acidic pH (5-6) where other FQs lose activity. FIN is intended for therapy of bacterial infections associated with an acidic environment such as *H. pylori* (HP) eradication and UTI. Susceptibility testing of HP, a fastidious slow growing organism, is challenging. The Etest stable gradient method has been evaluated for HP testing and is recommended by the European HP Study Group. This study compared MIC testing of FIN with Etest and agar dilution (AD) at neutral pH using a collection of HP, including FQ resistant strains.

Methods: AD was done according to the CLSI method. For Etest, HP inoculum prepared in Mueller Hinton broth + 5% sera (3 Mc Farland) was streaked onto Mueller Hinton agar plates with 10% aged blood. Plates with Etest strips were then incubated under microaerophilic conditions at 35°C and read after 3 and 5 days. One QC strain and 36 clinical isolates were tested in quintuplicate and triplicates respectively, with both MIC methods.

Results: MIC ranges and agreement between Etest and AD (see table).

Acceptable inter-method MIC agreement was seen after 3 and 5 days of incubation. Resistance that manifested as hazy growth in inhibition ellipses was easier to detect with Etest. Results from repeat testing with both MIC methods and the reference strain showed good reproducibility (agreement >95% ± 2 dilutions).

Conclusions: Substantially equivalent results can be obtained for MIC testing of FIN with HP using Etest and the CLSI reference method. Etest with its wide concentration range and simplicity of use makes it a useful MIC tool for drug development purposes especially for the testing of newer agents targeted against *H. pylori*.

Incubation (days)	Agreement %		MIC µg/mL	
	± 1 dilution	± 2 dilutions	FQ-susceptible	FQ-resistant
3	89.5	95.4	0.012-0.125	2-32
5	84.2	95.4	0.016-0.25	2-32
ATCC 43504 MIC (µg/mL)	Etest 0.032-0.125		AD 0.064-0.25	

F1-2041

Comparative Inhibitory and Bactericidal Activities of Finafloxacin (FIN) and Ciprofloxacin (CIP) against Gram-Negative and Gram-Positive UTI-pathogens Under Physiological Conditions and at Varying pH-values

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Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass that exhibits improved *in vitro* activity at slightly acidic pH and is therefore intended for treatment of UTI. The antibacterial and bactericidal activities of FIN and CIP were compared in artificial urine medium which reflects the physiological conditions of pH, ionic strength and chemical composition, encountered *in vivo*.

Methods: The MICs of FIN and CIP were determined against 34 strains (*S. aureus*, *S. saprophyticus*, Enterobacteriaceae, *P. aeruginosa*, incl. CIP^{res} and ESBL producers) using CLSI methodology in cation adjusted Mueller-Hinton Broth (CAMHB) at pH 7.2 and 5.8 and in synthetic urine (pH 5.8). Bactericidal activity was determined against 10 strains (10⁶ CFU/mL) exposed to 1 x, 4 x and 16 x MIC. During the initial log-linear phase of CFU-decline single point kill rates ($k = -\ln(N/N_0)/t$) were calculated.

Results: FIN MICs were 1 - 3 dilutions lower at pH 5.8 compared to at pH 7.2, whereas CIP MICs increased by 1 - 3 dilutions at the lower pH.

In artificial urine (pH 5.8), FIN exhibited MICs similar to those obtained in CAMHB pH 7.2, whereas CIP MICs increased by 10 - >100-fold. On average, FIN MICs were 4 - 5 dilutions lower than CIP in artificial urine, regardless of Gram type or susceptibility profile. Bactericidal activities of both FIN and CIP (kill-rates normalised to concentration (basis 1mg/L)) demonstrate that FIN is about 2- to >20-fold more active than CIP in CAMHB or synthetic urine.

Conclusions: The bacteriostatic (MICs) and bactericidal activities (time kill curves) of FIN differ favourably from those of CIP under conditions mimicking UTIs. The activity of FIN in artificial urine is quantitatively and qualitatively different from that of CIP. These findings indicate that FIN may be effective in the treatment of UTIs.

F1-2042

Bactericidal Activity Of Finafloxacin (FIN) Against Difficult To Kill Growth Forms Of *Escherichia coli*

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Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQ lose activity. Therefore, FIN is intended for bacterial infections associated with an acidic environment. During infection, bacteria may exist as adherent populations and form persistent subpopulations. This study assessed the ability of FIN to kill these difficult to treat growth forms.

Methods: Adherent populations of *E. coli* C700 were grown on 0.45 μ M membrane filters perfused with BHI (pH 6.2) to a steady state of 10⁷ - 10⁸ CFU/mL of perfusate. FIN, ciprofloxacin (CIP), levofloxacin (LVX) or moxifloxacin (MXF) (all 5 μ g/mL) were then perfused for 3d, followed by 1d of drug free media. Persistent subpopulations (persister frequencies) were defined as the fraction of viable cells that were recovered following exposure of high cell densities of *E. coli* ATCC 25922 (1 - 5 x 10⁸ CFU/mL) to FIN, CIP or LVX (all 10 μ g/mL, 24h) in Mueller-Hinton, pH 7.2 (MH) or artificial urine, pH 5.8 (AU).

Results: FIN resulted in a 5-log reduction of adherent *E. coli*, to below the limit of detection (<10² CFU/mL) within 5h, the nearest comparator to this was LVX (2-log reduction). All drugs had significantly reduced viability by day 3, however rapid regrowth was then observed following perfusion with drug-free media in the comparator-treated populations but no regrowth was observed after FIN treatment. The frequency of persisters that remained following high cell density killing in MH were FIN (5.2 x 10⁻⁷), CIP (1.6 x 10⁻³) and LEV (1.6 x 10⁻⁴). In AU medium these were FIN (1.2 x 10⁻⁴), CIP (4.1 x 10⁻³) and LVX (9.7 x 10³).

Conclusions: Adherent populations of *E. coli* were killed more rapidly by FIN and did not regrow following cessation of treatment, which was observed with the comparators. Such superior degree of killing may be related to the lower numbers of persistent bacteria isolated following exposure to FIN.

F1-2043

Selection and Characterisation of Finafloxacin, Ciprofloxacin and Levofloxacin Resistant Mutants of *Escherichia coli*

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Background: Finafloxacin (FIN) is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQs show decreased activity. Therefore, FIN is intended for therapeutic use against bacterial infections associated with an acidic environment. The *in vitro* emergence and genotypic mechanism of resistance to FIN, ciprofloxacin (CIP) and levofloxacin (LVX) was investigated in *E. coli* at pH 7.2 and pH 5.8.

Methods: Single-step mutants of *E. coli* ATCC 25922 were selected against FQ concentration gradients in Mueller-Hinton (MH) agar by plating 100 μ l of 1-3 x 10¹⁰ cfu/mL. MICs of stable mutants were determined by CLSI broth microdilution procedures at pH 7.2 and 5.8. Target mutation in gene segments of *gyrA* and *parC* of CIP, LVX and FIN resistant mutants were sequenced from PCR products. DNA sequences of mutants were aligned with parent.

Results: Resistance frequencies (4 x MIC) of first step mutants to FIN, CIP and LVX were 3.9 x 10⁻⁹, 2.1 x 10⁻⁹ and 4.2 x 10⁻⁹ respectively. First step mutants exhibited an 8 - 16-fold decrease in susceptibility over the parent and a similar decrease in susceptibility to the comparator FQs. All first step mutants (FIN, CIP & LVX) developed mutations within the quinolone resistance determination region (QRDR) of *gyrA*, the following substitutions were identified; G81D, S83L and D87N. No mutations in the QRDR of *parC* were detected.

Conclusion: FIN mutants arose at similar frequencies to the CIP and LVX mutants and exhibited similar decreases in susceptibility suggesting that FIN has the same low potential for resistance development. Mutations within the QRDR of *gyrA* were identified in FIN, CIP and LVX first step mutants of *E. coli* indicating this as a primary target.

F1-2044

Pharmacokinetics (PK) and *In Vivo* Efficacy of Oral Finafloxacin (FIN) and Comparators in Rodent Models of Systemic Infection

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Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQs show decreased activity. FIN was evaluated in comparison with several best in class FQs; ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF), in *in vivo* bacteraemia models with range of pathogens.

Methods: Serum concentrations were quantified from mice (3 / time point) by bioassay. Bacterial inocula were administered intraperitoneally, treatment commenced 0.5h postinfection and survival monitored over 3 - 5 days. Groups of 5 - 6 female CFW-1 mice or 5 Wistar rats were used. Additionally, treatment of *M. catarrhalis* colonisation was assessed by reduction of bacterial counts ($\Delta \log_{10}$ CFU/mL) in multiple tissues.

Results: The following PK parameters (normalised to 1mg/kg) were determined for **FIN**, MXF, CIP, LVX following oral administration; AUC [kg*h/L] (**0.57**, 0.15, 0.1, 0.22), C_{max} [kg/L] (**0.36**, 0.17, 0.04, 0.18), t_{1/2} [h] (**1.52**, 1.26, 1.84, 0.59). The minimum protective oral (or i.v.) doses (i.e. 100% survival) of **FIN**, MXF, CIP, LVX (all mg/kg) in the following bacteraemia models were; *S. aureus* (**10**, 25, 25, >25), MRSA CIP^{res} (**50**, 50, >50, >50), *E. faecalis* (**1**, >25, >25, 25), VRE (**10**, 10, 25, 25), *S. pneumoniae* (**25**, 25(MXF)), *S. pneumoniae* PEN^{res} (**25**, 50, >50, 50), *S. pyogenes* (**50**, 50, >50, >50), *E. coli* (**0.5**, 10, 1, 10), *S. marcescens* (i.v., **5**, 5, 0.2, 1), *K. pneumoniae* (**>2.5**, 1, 2.5, 2.5), *S. pneumoniae* (rat) (**25**, >25, >25, >25) and *L. monocytogenes* (**25**, >25, >25, >25). FIN (10mg/kg p.o.) was considerably more active than the other FQs in reducing the viable load of *M. catarrhalis* from the lungs of colonised mice, exhibiting a $\Delta \log_{10}$ CFU/mL of >-3.

Conclusions: FIN compared to MXF, CIP and LVX exhibited comparative or, in most cases, superior efficacy in rodent bacteraemia models with an extensive range of pathogens.

F1-2045

***In Vivo* Efficacy of Finafloxacin (FIN) in Difficult to Treat Animal Models of Infection**

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Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass that exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQ show decreased activity. FIN was evaluated along with ciprofloxacin (CIP), levofloxacin (LVX) and moxifloxacin (MXF), in a wide range of *in vivo* models.

Methods: Female CFW-1 mice (n = 6) were used. Bacterial inocula were administered by the following routes: intraperitoneal, oral application, implantation of colonised catheter material or direct injection into the kidney, bladder, thigh, granuloma pouch or abscess. Treatment was commenced 0.5 - 3h postinfection. End points were determined by % survival (at 3 - 5 days) or by reduction of bacterial counts ($\Delta \log_{10}$ CFU/mL) in homogenised tissue.

Results: FIN (10mg/kg s.c.) exhibited greater killing of *S. aureus* in the thigh muscle ($\Delta \log_{10}$ CFU/mL: FIN -4, MXF -3, CIP and LVX both -2) than the comparators.

FIN (10mg/kg p.o.) exhibited greater killing of *S. aureus* ($\Delta \log_{10}$ CFU/mL: FIN -2, MXF -1) and of *P. aeruginosa* ($\Delta \log_{10}$ CFU/mL: FIN -1, CIP, LVX and MXF all <(-1)) than the other FQs in infected abscess models. FIN (10mg/kg p.o.) exhibited bactericidal activity in severe *E. coli* pyelonephritis ($\Delta \log_{10}$ CFU/mL; FIN -4, CIP -3, LVX -4, MXF -3) and also in ascending *P. mirabilis* cystitis ($\Delta \log_{10}$ CFU/mL; FIN and CIP (100mg/kg p.o.) -4). Additionally, FIN exhibited equal, if not superior activity to the comparators in granuloma pouch and implanted catheter models (*S. aureus*), enteritis (*S. typhimurium*), and post surgical polymicrobial peritonitis.

Conclusion: The superior efficacy of FIN over CIP, LVX and MXF in these difficult to treat models was in line with their respective MICs at lower pH values which are anticipated to occur in many infection models, especially those involving inflammation (abscess) or low pH fluids such as urine. These data suggest that the pH activity profile of FIN may be an advantage in combating severe bacterial infection.

F1-2046

Comparative Activity Between Finafloxacin (FIN) and Other Fluoroquinolones Against Bacterial and Eukaryotic Type II Topoisomerases

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Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at a slightly acidic pH (5.0 - 6.0) under which other FQ show decreased activity. Because of this property, FIN is intended for therapeutic use against bacterial infections associated with an acidic environment. The selectivity of FIN for eukaryotic and bacterial DNA topoisomerase II enzymes was evaluated using quantitative plasmid DNA cleavage assays *in vitro*.

Methods: The ability of FIN, clinafloxacin (CLX), ciprofloxacin (CIP), moxifloxacin (MXF) and enoxacin (ENX) to induce DNA cleavage from human topo II α , *E. coli* DNA gyrase and topo IV was quantified and compared based on the cleavage detection limit (CDL), defined as the lowest concentration yielding detectable cleavage product compared with that of the known topo II poison, etoposide (VP16). The CL₅₀ value, defined as the concentration that induces 50% maximum cleavage, was used as an additional endpoint for bacterial enzymes.

Results: The activity of FIN against the human enzyme was 250-fold lower than that of VP16 and places it well amongst the other FQ (in terms of fold lowered activity against the human enzyme) *viz*; CLX (10 - 50), CIP (100 - 250), MXF (500) and ENX (no CDL detectable). FIN, CLX, CIP and MXF exhibited a CDL of 1ng/mL against bacterial DNA gyrase, ENX exhibited lower activity (10ng/mL). FIN, CLX and MXF displayed comparable activity against topo IV (1ng/mL), while CIP (10ng/mL) and ENX (50ng/mL) were less active. CL₅₀ (ng/mL) against gyrase and topo IV respectively show that FIN (25, 8) was more active against both bacterial targets than CLX (10, 52), CIP (120, 200), MXF (70, 200) and ENX (50, 500).

Conclusions: These data indicate that FIN is highly selective for bacterial type II topoisomerases. FIN exhibited superior activity to the comparator FQs in terms of potency against the individual bacterial enzymes and its relative equipotency against these dual targets.

F1-2047

In Vitro Toxicological Profiling of Finafloxacin (FIN)

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Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQs show decreased activity and is therefore intended, e.g., for treatment of *H. pylori* and UTI. Several novel FQs have recently failed during development or shortly after launch due to safety / toxicology concerns. Therefore, prior to start of formal GLP safety / tox studies, FIN was rigorously profiled for the most common class related side effects against a series of predictive *in vitro* tests alongside comparator FQs; Ciprofloxacin (CIP), Trovafloxacin (TRO), and Sparfloxacin (SPA) and standard test controls, where appropriate.

Methods: Cytotox potential was determined against the mouse macrophage line J774.AI and phototox potential against the mouse fibroblast Balb/c 3T3 following exposition to UV irradiation. Extracellular recordings from slices of rat hippocampus were used to investigate excitatory and neurotox potential, chondrotox potential was studied on primary cartilage cells from dog and man and hepatotox potential on primary rat hepatocytes. Seizure potential was studied on GABA-A receptors from the ileum of guinea pigs and cardiotox (incl. QT effects) in Langendorff heart preparations and hERG channel experiments.

Results: (see table)

Conclusions: FIN was examined in a series of *in vitro* cell based assays that are believed to be predictive of the most common, FQ-associated undesirable side effects. Under the conditions of these assays, FIN displayed a profile indicative of a low potential for the toxicity issues often associated with FQs.

Test system	Finafloxacin result	Comparator result
Cytotox mouse MΦ J774.AI	EC ₅₀ = 100 µg/mL	-
Phototox mouse fibroblasts 20'/1h radiation	EC ₅₀ > 100 / 100 µg mL	EC ₅₀ > 100 / = 60 µg/mL (CIP)
Hippocampus slice test	98 % of control	276 % of control (TRO)
Primary hepatocytes	NOEC ³ 100 µg/mL	NOEC 10 µg/mL (TRO)
Primary dog chondrocytes	NOEC ³ 100 µg/mL	NOEC = 10 µg/mL (CIP)
Primary human chondrocytes	NOEC ³ 100 µg/mL	NOEC = 30 µg/mL (CIP)
GABA-A receptor	NOEC > 100µM	0.3 µM ((+)-bicuculline)
Langendorff heart preparation	NOEC > 300 µM	-
hERG channel activity	NOEC > 300 µM	ED50 ~ 25 µM (SPA)

F1-2048

A Phase I Study to Determine Safety, Tolerability and Pharmacokinetics (PK) of Finafloxacin (FIN) in Healthy Subjects

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Background: FIN is a novel fluoroquinolone (FQ) under early clinical development. FIN exhibits optimal activity at slightly acidic pH (pH 5.0 - 6.0). A combined Phase I study protocol was designed to evaluate safety, tolerability and PK of single and multiple ascending oral doses of FIN in healthy adult subjects.

Methods: The study was designed as a single-center, inpatient, double-blind, randomized, placebo-controlled, not weight-adjusted, single and multiple escalating dose study of FIN oral tablets. 75 (64 males, 11 females) subjects were included, 3 of which received a single dose of 25 mg, 40 of which (in groups of 6+2) received single doses of 50 - 800 mg FIN/placebo under fasting conditions. A further 32 were given doses of 150, 300, 600 or 800 mg for 7 consecutive days. Laboratory safety assessment, vital signs and ECGs were evaluated. Plasma and urine samples for the determination of the PK were collected over a period of 48h post dose.

Results: All enrolled subjects completed the study. No relevant changes in laboratory test parameters were observed. Adverse events were recorded for 35 of the 75 subjects including (but not limited to): headache (11 incidents), tiredness (10), feeling of pressure in the head (7), diarrhoea (5) and nausea (3). No serious adverse events were reported. At 400 and 800 mg single doses the plasma $t_{1/2}$ of FIN was 9.3 and 10.2h, C_{max} [$\mu\text{g/mL}$] was 4.6 and 9.1; and $AUC_{0-tlast}$ [$\text{hr}\cdot\mu\text{g/mL}$] was 13.3 and 23.9 respectively. FIN was readily absorbed with peak plasma concentration achieved at 0.25-2h after dosing. The systemic exposure ($AUC_{0-tlast}$) of FIN increased linearly from 25 to 800 mg. For 400 mg and 800 mg, mean urinary excretion was 28.3 % and 33.4 %, respectively.

Conclusions: Single and multiple doses were very well tolerated at all evaluated doses. Based on the good safety, tolerability and PK profile, FIN warrants further clinical evaluation.

F1-2049

Urinary Pharmacokinetics and Bactericidal Activity of Finafloxacin (FIN) (800mg) in Healthy Volunteers Receiving a Single Oral Dose

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Background: FIN is a novel fluoroquinolone (FQ) belonging to a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH (5.0 - 6.0), under which other FQs lose activity. Therefore, FIN is intended for bacterial infections associated with an acidic environment (such as UTI). This study assessed the urinary pharmacokinetics (PK) and bactericidal activity of FIN in 6 healthy volunteers receiving a single, 800 mg oral dose.

Methods: Urinary concentrations were determined over a 24h period. Urinary bactericidal titers (UBTs) were defined as the highest dilution of subject urine (following dilution in antibiotic free urine) that exhibited bactericidal activity. UBTs were determined at intervals over 24h to produce an area under the 24h UBT dilution steps-time-curve (AUBTs) for FIN in native and acidified urine against 1 test strain (E.coli ATCC 25922) and 6 ciprofloxacin (CIP) susceptible uropathogens.

Results: The mean (median) maximum concentration of FIN in urine was 150 (137) mg/L at 4 to 8 hours. Median UBT (0-4h) and AUBT 24h [h⁻¹] of FIN against 1 test strain and 6 uropathogens (**siehe nächste Folie**).

Conclusions: FIN (800mg) exhibits bactericidal activity in ex vivo urine against a range of UTI pathogens and warrants further investigation for this indication.

F1-2049 (Forts.)

Strain	CIP MIC (in CAMH broth) pH 7.2 (µg/mL)	CIP MIC (in artificial urine pH 5.8) (µg/mL)	FIN MIC (in artificial urine pH 5.8) (µg/mL)	UBT* (0-4h)	AUBT* [h ⁻¹] 24h native urine	AUBT* [h ⁻¹] 24h urine pH 5.5
<i>E. coli</i> ATCC25922	£0.004	1	0.03	>2048	41,984	35,840
<i>E. coli</i> #523 (NR)	0.06/0.03	16	2	384	2,944	3,264
<i>E. coli</i> MI-4	1/0.5	128	4	48	504	488
<i>K.</i> <i>pneumoniae</i> #95	≤0.125	2	0.25	512	9,472	8,448
<i>P. mirabilis</i> #414	≤0.004	2/1	4	32	448	552
<i>P.</i> <i>aeruginosa</i> #568	0.25	8	2	48	568	776
<i>E. faecalis</i> #60	2	16	2	64	624	360

F1-2052

In Vitro Activity of JNJ-Q2, a New Fluoroquinolone, against Susceptible and Multi-Drug Resistant *Streptococcus pneumoniae*

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Background: JNJ-Q2, a new fluoroquinolone, has in vitro antibacterial activity against Gram-negative and Gram-positive organisms, including levofloxacin (LVX)-resistant *S. pneumoniae* (Spn). The activities of JNJ-Q2 were examined against clinical Spn isolates, including those with mutations in the quinolone-resistance determining region (QRDR).

Methods: Broth microdilution MICs (CLSI methodology) were determined against 98 clinical Spn isolates, including erythromycin (ERY)-resistant and penicillin (PEN)-nonsusceptible isolates. 20 recent clinical quinolone-resistant Spn isolates with mutations in DNA gyrase and topoisomerase IV were tested. Time-kill kinetics were determined (CLSI methodology).

Results: JNJ-Q2 displayed potent in vitro activity against 98 clinical *S. pneumoniae*, with an MIC₉₀ of 0.06 µg/mL, 64-fold more potent than moxifloxacin (MOX). The MIC₉₀ of JNJ-Q2 was ≤0.12 µg/mL against subsets of ERY-R, PEN-NS or LVX-R *S. pneumoniae* isolates. Independent clinical Spn isolates carrying QRDR mutations in both *parC* and *gyrA* displayed JNJ-Q2 and MOX MICs ranging between 0.06 to 0.5 µg/mL and 2 to 16 µg/mL, respectively. JNJ-Q2 exhibited bactericidal activity in time-kill studies, reducing the *S. pneumoniae* inoculum >3 log₁₀ units in 8 hours at 2X MIC.

Conclusions: JNJ-Q2 displayed potent in vitro activity against recent *S. pneumoniae* isolates, including multidrug-resistant isolates non-susceptible to penicillin, erythromycin and/or levofloxacin. Spn isolates with characterized QRDR mutations, including those with Ser79 substitutions in ParC and Ser81 substitutions in GyrA had JNJ-Q2 MICs ≤0.25 µg/ml

Organism	Agent	MIC Range	MIC ₅₀	MIC ₉₀
<i>S. pneumoniae</i> (n=98)	JNJ-Q2	≤0.004 - 0.25	0.015	0.06
<i>S. pneumoniae</i> (n=98)	Moxifloxacin	0.12 - 16	0.25	4
<i>S. pneumoniae</i> (n=98)	Penicillin	≤0.008 - 4	0.06	2
<i>S. pneumoniae</i> (n=98)	Erythromycin	0.03 - >16	0.06	>16
<i>S. pneumoniae</i> QRDR Mutants (n=20)	JNJ-Q2	0.06 - 0.5	0.12	0.25
<i>S. pneumoniae</i> QRDR Mutants (n=20)	Moxifloxacin	1 - 16	4	8

F1-2053

Antistaphylococcal Activity of NXL 101 Compared to Other Agents

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Background: MRSA strains are therapeutic problems all over the world, and life-threatening infections caused by CA-MRSA strains are increasingly found. MRSA strains which are not glycopeptide susceptible have appeared in many locations. NXL 101 is a new compound predominantly active against *gyrA*. We describe activity of NXL 101 compared to vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, levofloxacin, moxifloxacin against a spectrum of MRSA.

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

Results: Twenty-eight strains showed inducible clinda resistance (8 community-, 11 hospital-acquired, 9 vancomycin non-susceptible). MIC₅₀ and MIC₉₀ values (µg/ml) were as follows (see table). NXL 101 had excellent anti-staphylococcal activity with an MIC range (µg/ml) 0.06-0.5, and MIC₅₀ and MIC₉₀ values (µg/ml) of 0.125, 0.25, respectively, against all strains regardless of phenotype. All strains were susceptible to linez and tige and all except 21 strains (all VISA)(MICs 2-4 µg/ml) were dapto susceptible. Quinolone resistance was only found amongst hospital acquired and vanco non-susceptible strains.

Conclusions: NXL 101 had excellent activity against 200 MRSA strains, with MICs (µg/ml) ranging between 0.06-0.5.

Drug	Community-acquired (127)			Hospital-acquired (40)			hVISA+VISA+VRSA (33)		
	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90
NXL 101	0.06-0.5	0.125	0.25	0.06-0.25	0.125	0.25	0.06-0.5	0.125	0.25
Vancomycin	0.5-1	1	1	0.5-2	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

F1-2054

Optimization of the Anti-MRSA Activity of 3-Aminomethylpyrrolidine-Substituted Isothiazoloquinolones

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Background: Optimization of isothiazoloquinolones (ITQs) for potency against multi-drug resistant *S. aureus* has previously defined the optimal tricyclic core construct and its substituents. The 7-position was further optimized for anti-MRSA activity, specifically altering substitution patterns and stereochemistry of a 3-aminomethylpyrrolidine group.

Methods: Stereospecific synthesis of substituted 3-aminomethylpyrrolidines was used to create a series of related ITQ analogs. Antibacterial activities and general cytotoxicity were assessed along with target enzyme (gyrase and topoisomerase IV) activity of select compounds using standard methodologies.

Results: Most analogs were highly active against *S. aureus* and *E. coli*. Potency was generally diminished by N-substitution on nitrogen and improved with substitutions alpha to the terminal nitrogen. The R-configuration of the 3-position of the pyrrolidine was optimal for MRSA potency. Many of these analogs had MICs below 1 ug/mL against quinolone-resistant MRSA. This could be attributed to the increased effectiveness against wild-type and mutant *S. aureus* gyrase relative to quinolones.

Conclusions: Optimization of substitution patterns of 7-(3-aminomethylpyrrolidine)-ITQs lead to exceptionally potent compounds against

F1-2055

Systemic Hypersensitivity Test of Nemonoxacin, a Novel Potent Broad-Spectrum Non-Fluorinated Quinolone, in Guinea Pigs

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Background: Nemonoxacin is a novel non-fluorinated quinolone (NFQ) effective against multi-drug resistant infections, including methicillin-resistant *Staphylococcus aureus* (MRSA). The purpose of this study was to evaluate the systemic hypersensitivity potential of Nemonoxacin when administered to guinea pigs.

Methods: Guinea pigs (3/sex) were randomly assigned to 4 groups receiving intraperitoneal injections of negative control (0.9% normal saline), positive control (bovine serum albumin), and Nemonoxacin at 10 and 20 mg/kg. The study consisted of induction and challenge phases. In the induction phase, animals were dosed every other day for a total of 5 injections. In the challenge phase, animals received 2-fold dosages of the induction doses intravenously on Day 10 after the last induction dose. During the induction phase, animals were observed daily and weighed on the first and last induction days and 1 day before challenge initiation. In the challenge phase, animals were observed for possible systemic hypersensitivity reactions and their onset/offset times.

Results: During the induction phase, no Nemonoxacin-related clinical signs of toxicity or mortality were observed. There were no significant differences in body weight changes among all tested groups. In the challenge phase, no hypersensitivity signs were noted in the negative control or Nemonoxacin-treated groups. In contrast, five out of six positive control animals developed “very strong hypersensitive reaction”, i.e., death, with the remaining animal showing a “strong hypersensitive reaction”, e.g., dyspnea, rattle, gasping, cartwheel, and/or tidal respiration, etc.

Conclusions: Based on the above results, it was concluded that Nemonoxacin did not demonstrate any sensitization potential and would not pose any allergenic/sensitizing risks in patients.

F1-2056

Fertility and Early Embryonic Developmental Toxicity of Nemonoxacin after Oral Administration to Rats

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Background: Nemonoxacin is a novel non-fluorinated quinolone effective against multi-drug resistant infections, including methicillin-resistant *Staphylococcus aureus* (MRSA). The purpose of this study was to evaluate the fertility and developmental (Segment I) toxicity of Nemonoxacin after oral administration to male and female rats.

Methods: This study followed the ICH Harmonized Tripartite Guideline Stages A and B of the reproductive process. Sprague-Dawley rats (25/sex/group) were given with Nemonoxacin orally by gavages at dose levels of 0, 30, 300 and 1000 mg/kg. Male rats were dosed once daily beginning 28 days before cohabitation and continuing through the day before sacrifice; female rats were dosed once daily beginning 14 days before cohabitation and continuing through day 7 of presumed gestation. Rats were observed for mortality, clinical signs, body weights and food consumption, and examined grossly. Weight and histology of reproductive organs, reproductive performance, estrous cycles, and semen were also analyzed. Female rats were examined for numbers of *corpora lutea*, implantation sites, viable and non-viable embryos, and pre- and post- implantation losses.

Results: There were no clinical signs and mortality in this study. Significant decreases in body weight gain and food consumption were observed during gestation and in the first dosing week, respectively, in the mid- and high-dose female rats. There were decreases in absolute and relative weights of epididymis and prostate in the mid- and/or high-dose male rats. These changes were not considered to be toxicologically significant because neither semen changes nor histopathological findings were observed in these organs.

Conclusions: Oral administration of Nemonoxacin did not produce any fertility or reproductive adverse effects under the experimental conditions of this study. The no observable adverse effect levels (NOAELs) of Nemonoxacin for maternal and developmental toxicities in the rats were 30 and 1000 mg/kg/day, respectively.

F1-2057

Activity of Nemonoxacin, an Investigational C8-methoxy Non-fluorinated Quinolone Against Gram-Positive Cocci Obtained From Canadian Hospitals: CANWARD 2007

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Background: Nemonoxacin (Nemo) is a novel C8-methoxy non-fluorinated quinolone. The purpose of this study was to assess the activity of Nemo against gram-positive cocci obtained from Canadian hospitals as part of the CANWARD 2007 study.

Methods: From Jan-Dec 2007, 12 hospitals across Canada submitted isolates from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units. 7881 isolates were collected including 3473 gram-positive cocci. Susceptibility testing was performed using CLSI broth microdilution.

Results: MIC₅₀ and MIC₉₀ values for Nemo and levofloxacin (Levo) are below **Median MIC.

Conclusion: Nemonoxacin is more active invitro than levofloxacin against gram-positive cocci including SPN, MSSA, MRSA, VISA, VRSA, MSSE, MRSE and *E. faecalis*.

Organism (# isolates)	Nemo MIC ₅₀ /MIC ₉₀	Levo MIC ₅₀ /MIC ₉₀
SPN-All (656)	0.015 / 0.015	0.5 / 1
- PenS (519)	0.015 / 0.015	0.5 / 1
- PenI (103)	0.015 / 0.015	0.5 / 1
- PenR (34)	0.015 / 0.03	0.5 / 2
- CipR (29)	0.03 / 0.12	2/16
MSSA (372)	0.03 / 0.12	0.25 / 4
CA-MRSA (23)	0.25 / 0.5	4 / 8
HA-MRSA (91)	4/>4	>32 / >32
MSSE (32)	0.03 / 0.5	4 / >32
MRSE (9)	2 / 2	>32 / >32
<i>E. faecalis</i> (81)	0.12 / 1	2 / >32
*VISA (12)	1/2	32 / >32
*VRSA (7)	2**	32**

L-678

Efficacy and Safety of Nemonoxacin versus Levofloxacin for the Treatment of Community-Acquired Pneumonia

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Background: Community-acquired pneumonia (CAP) is a major cause of morbidity and mortality worldwide. This study compared the efficacy and safety of nemonoxacin (Nemo, orally 500 mg or 750 mg once daily) to that of levofloxacin (Levo, orally 500 mg once daily) for 7-day treatment of CAP.

Methods: A Phase II, randomized, double-blind, comparative, multi-center study in adults with CAP. The primary end point was clinical cure rate at the test-of-cure (TOC) visit. The bacteriologic success rate at TOC visit was assessed in subjects with a baseline pathogen.

Results: This study met the clinical efficacy endpoints in intent-to-treat, per-protocol, and clinically evaluable populations. A total of 265 subjects were randomized to 3 treatment arms at a 1:1:1 ratio. The clinical cure rates in the clinically evaluable population were 92% (66/72) for Nemo 750-mg, 88% (64/73) for Nemo 500-mg, and 90% (65/72) for Levo 500-mg. Nemo showed non-inferiority to Levo in clinical outcome with 2-sided 97.5% CI. In the bacteriologically evaluable population, the bacteriologic success rates were 92% (34/37) for Nemo 750-mg, 84% (37/44) for Nemo 500-mg, and 94% (44/47) for Levo 500-mg. It was noted that C_{max} and AUC_{0-24} of Nemo 500-mg were lower than those of Levo 500-mg. The most common isolates included *H. influenzae*, *S. pneumoniae*, and *S. aureus*. There was no significant difference between 3 treatments in bacteriological outcomes by pathogen. All 3 treatments were well tolerated with no drug-related serious AEs. No clinically significant difference of drug-related AEs was noted between 3 treatments. Nemo demonstrated the consistent favorable safety profile as observed in previous clinical trials.

Conclusions: Nemonoxacin 750 mg once daily is as effective and well tolerated as levofloxacin 500 mg once daily, over a 7-day course in the treatment of adults with CAP.