Expert Opinion on Investigational Drugs

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Meeting Highlights

40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)

Toronto, Canada, September 17 - 20, 2000

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The 40th ICAAC Meeting provided one of the widest varieties of new antibacterial and antifungal agents in years, with a range of improved 'classical' agents (β-lactams, carbapenems), novel quinolone agents and totally new ideas for novel therapeutic intervention. Among the compounds reported were potent anti-MRSA carbapenems, novel des-fluoroquinolones (BMS 284756/T-3811), non-fluorinated quinolones, quinolone-related antibacterials, novel cephalosporins (cefditoren, RWJ-54428, MC 04546), ketolides (telithromycin, ABT-773), novel streptogramins and novel β-lactamase inhibitors, bacterial and fungal efflux pump inhibitors and novel antifungals (azasordarins, echinocandins, azoles).

Keywords: *β-lactamase, antibacterials, antifungals, cephalosporins, efflux pump inhibitors, ketolides, quinolones*

Exp. Opin. Invest. Drugs (2000) ***9****(12):2945-2972*

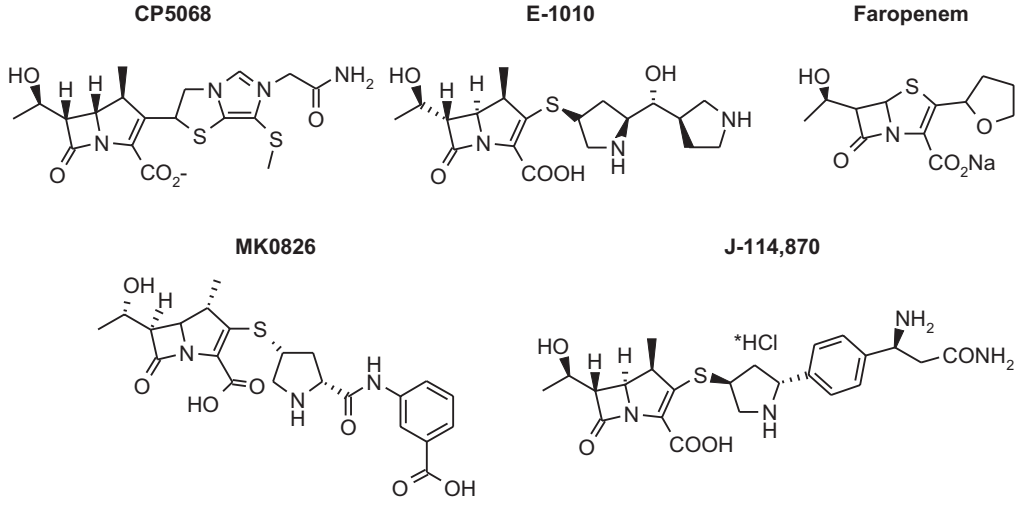
1. Carbapenems

1.1 CP5068

CP5068 (Meiji Seika Kaisha Ltd., Yokohama, Japan) (**Figure 1**), a novel carbapenem with potent anti-MRSA activity, possesses methicillin-resistant *Staphylococcus epidermidis* (MRSE) and methicillin-resistant *Staphylococcus aureus* (MRSA) activity nearly equivalent to vancomycin (E Shitara *et al.*, Meiji Seika Kaisha Ltd.) with an MIC_{90} of 4 μg/ml against MRSA and 4 μg/ml against MRSE (T Ida *et al.*, Meiji Seika Kaisha Ltd.). CP5068 has a 6,7-disubstituted imidazo [*S*, 1-β] thiazolium-2-yl group on the C-2-position of the carbapenem nucleus.

Specifically designed to target PBP2′ of MRSA, CP5068 exhibited 124-times greater affinity than imipenem to *S. aureus* strain MF535HR (MRSA) with an IC_{50} to PBP2′ binding of 1.7 μg/ml (*vs.* IC_{50}, 210 μg/ml for imipenem) (T Ida *et al.*). CP5068 is more bactericidal than cefotaxime or penicillin G against highly penicillin-resistant *Streptococcus pneumoniae* (PRSP) (K Ubukata *et al.*, Inst. Microb. Chem., Tokyo, Japan). In direct comparison with other penems, CP5068 was 16-times more active than imipenem, 32-times more active than meropenem and four-times more active than panipenem and CP5068 was 128-times more active than cefotaxime, 64-times more active than levofloxacin and 16-times more active than vancomycin against PRSP

Figure 1: Chemical structures of carbapenems.



and penicillin-intermediate *S. pneumoniae* (K Ubukata *et al.*). In a PRSP systemic infection model in mice, CP5068 was 14-times more potent (ED_{50}, 0.16 mg/kg/day) than meropenem (ED_{50}, 2.22 mg/kg/day), three-times more potent than panipenem (ED_{50}, 0.44 mg/kg/day), 78-times more potent than ceftriaxone, 142-times more potent than cefotaxime (ED_{50}, 22.8 mg/kg/day) and 23-times more potent than vancomycin (ED_{50}, 3.72 mg/kg/day) (K Ubukata *et al.*). CP5068 demonstrated excellent potency in a PRSP experimental meningitis model in rabbits, with a > 3 log reduction in CFU at 20 mg/kg. In the same model, the $t_{½}$ was 0.29 h, $AUC_{O\rightarrow\infty}$ = 13.8 mg.h/l (in plasma) and $AUC_{O\rightarrow\infty}$ = 4.6 mg.h/l (in CSF). Scaling predictions suggest a b.i.d. dosing in man.

PK parameters of CP5068 in mice for a single dose, 44 mg/kg, resulted in a C_{max} of 80.1 μg/ml, $t_{½}$ of 12.3 min and $AUC_{O\rightarrow\infty}$ 38.7 mg.h/l in serum and a C_{max} of 27 μg/ml, $t_{½}$ of 32.3 min and $AUC_{O\rightarrow\infty}$ of 14.6 μg.h/gm of lung tissue (T Ida *et al.*). Protein binding to serum protein for CP5068 was 19.8% in mice and 7.5% in humans (E Shitana *et al.*, Meiji Seika Kaisha Ltd.). CP5068 exhibited the highest relative stability to renal DHP-1, as compared with meropenem, panipenem and imipenem (E Shitara *et al.*).

CP5068 minimal lethal dose in mice was over 2000 mg/kg, with $GABA_A$ receptor binding in rats showed CP5068 similar to panipenem, better than meropenem and less safe than imipenem. No kidney nephrotoxicity by CP5068 was observed in rats (E Shitara *et al.*).

1.2 J-114,870

J-114,870 (**Figure 1**), a new 1-β-methycarbapenem with a C-2-position trans-3,5-disubstituted 5-arylpyrrolidin-3-ylthio moiety, is a broad spectrum carbapenem with excellent activity against MRSA (MIC_{90}, 4 μg/ml), PRSP (MIC_{90}, 0.125 μg/ml) and *Pseudomonas aeruginosa* (MIC_{90}, 4 μg/ml) (K Shibata *et al.*, Tsukuba Res. Inst., Banyu Pharm. Co. Ltd., Tsukuba, Japan).

Efficacy of J-114,870 against MRSA and *P. aeruginosa* mouse septicaemia models, was with an ED_{50} of 5.49 mg/kg, as compared with imipenem (ED_{50} 74.35 mg/kg) or vancomycin (ED_{50}, 4.25 mg/kg) against MRSA and ED_{50} values for J-114,870, imipenem and vancomycin of 0.16, 0.32 and > 100 mg/kg, respectively, against *P. aeruginosa* (K Shibata *et al.*). Superior *in vivo* potency by J-114,870 was also demonstrated against PRSP, with ED_{50} values of 0.12, 0.15, 0.29 and 5.85 mg/kg against J-114,870, imipenem, meropenem and penicillin G, respectively (K Shibata *et al.*). J-114,870 demonstrates equal bactericidal activity to other penems (meropenem, imipenem).

1.3 E-1010

E-1010 (**Figure 1**), a parenteral 1-β-methyl carbapenem with broad spectrum antibacterial activity in development by Eisai Co. Ltd. (Tokyo, Japan). T Hirawawa *et al.* (Eisai Co. Ltd.) reported on Phase I double-blind, placebo-controlled, ascending, single-dose study in healthy males, with doses ranging from 250 - 1000 mg. PK parameters for the 250 mg dose

were C_{max}, 19.7 μg/ml, $AUC_{O\rightarrow\infty}$ 37.4 mg.h/l, V_{ss}, 0.18 l/kg, $t_{½}$, 1.72 h and CL, 0.09 l/h/kg, while the 1000 mg dose had C_{max}, 66 μg/ml, $AUC_{O\rightarrow\infty}$ 131.5 mg.h/l, V_{ss}, 0.21 l/kg, $t_{½}$, 1.79 h and CL 0.10 l/h/kg (T Hirasama *et al.*). All 41 volunteers initiated and completed this Phase I trial. The plasma elimination $t_{½}$ of E-1010 (1.7 - 1.8 h) was longer than meropenem or imipenem (~ 1 h). Urine was the major elimination route of E-1010, with 60 - 76% of dose identified within 24 h of dosing. Imipenem-susceptible *P. aeruginosa* (MIC values, 1.0 μg/ml) and imipenem-resistant *P. aeruginosa* (MIC values, 16 μg/ml) were found to be susceptible to E-1010, with MIC values of 0.25 and 1.0 μg/ml, respectively (T Toyosawa and M Inoue, Kitasata Univ. Sch. Med., Kanagawa, Japan). Using these strains in an *in vitro* pharmacodynamic model simulating human plasma levels at b.i.d. dosing with E-1010 or imipenem, *in vivo P. aeruginosa* potency was determined. E-1010 demonstrated high level killing in 5 h (99.9% killing), with a maximum 3.5 logs killing overall. Imipenem, however, did not reach 99.9% killing and overall had a maximum killing of 2.5 logs against imipenem-resistant *P. aeruginosa* (T Toyosawa and M Inoue).

1.4 MK0826

MK0826 (ertapenem, L-749,345; Merck Pharm. Co., Rahway, NJ) (**Figure 1**) is a novel, parenteral, long-acting 1-β-methyl carbapenem with broad spectrum activity, including anti-pneumococcal and anti-anaerobic activity (GA Pankoch *et al.*, Hershey Med. Center, Hershey, PA; KM Overbye *et al.*, Merck Res. Laboratories, Rahway, NJ). Against extended spectrum β-lactamases (ESBLs) producing organisms, MK0826 had an MIC_{90} of 0.125 μg/ml, as compared with ceftriaxone (MIC_{90}, 64 μg/ml) and piperacillin/tazobactam (MIC_{90}, 128 μg/ml) (KM Overbye *et al.*, Merck Res. Laboratories). The anti-pneumococcal activity ($MIC_{50/90}$s) of MK0826, amoxicillin, amoxicillin/clavulanate, cefuroxime, cefprozil, cefepime, ceftriaxone, imipenem, meropenem and clarithromycin were 0.5/1.0, 2/2, 2/2, 8/8, 16/16, 1/2, 1/2, 0.25/0.25, 0.5/1 and > 32/32 μg/ml, respectively, against penicillin-resistant, quinolone-sensitive strains of *S. pneumoniae* and 0.125/1, 0.25/2, 0.25/2, 0.5/8, 0.5/16, 0.25/1, 0.25/1, 0.03/0.25, 0.06/0.5 and ≤ 0.03/8 μg/ml, respectively, for penicillin-resistant, quinolone-resistant strains of *S. pneumoniae* (GA Pankuch *et al.*). MK0826 has excellent anti-*Haemophilus influenzae* activity with $MIC_{50/90}$s of 0.03/0.06 and 0.03/0.06 μg/ml against β-lactamase-negative and β-lactamase-producing *H. influenzae*, respectively, as compared with penicillin, amoxicillin/clavulanate and cefuroxime with $MIC_{50/90}$s of 0.25/1, 0.25/1 and 0.5/1 μg/ml, respectively, *versus* β-lactamase-negative *H. influenzae* and > 8/ > 8, 1/1 and 0.5/1 μg/ml, respectively, against β-lactamase-producing *H. influenzae* (GG Zhanel *et al.*, Univ. of Manitoba, Winnipeg, Manitoba, Canada). Likewise, MK0826 had excellent activity against *Moraxella catarrhalis*, with $MIC_{50/90}$s of ≤ 0.015/≤ 0.015, 8/16, 0.25/0.5, 1/2 and 0.25/0.5 μg/ml for MK0826, penicillin, amoxicillin/clavulanate, cefuroxime and cefotaxime, respectively (GG Zhanel *et al.*). Against a panel of 431 clinical isolates of anaerobes including *Bacteroides fragilis*, *Prevotella*, *Porphyromonas* and *Fusobacterium*, imipenem = meropenem ($MIC_{50/90}$s 0.12/1 μg/ml) > MK0826 ($MIC_{50/90}$s, 0.25/2 μg/ml) > clindamycin = piperacillin/tazobactam ($MIC_{50/90}$s, 0.5/16 μg/ml) > metronidazole ($MIC_{50/90}$s, 1/ > 16 μg/ml) > ceftriazone ($MIC_{50/90}$s, 16/ > 64 μg/ml) (LM Kelly *et al.*, Hershey Med. Ctr., Hershey, PA); 95.8% of anaerobes tested were susceptible at a proposed breakpoint of ≤ 4 μg/ml for MK0826. The MIC_{50}/MIC_{90} value for MK0826 against all strains tested was 0.25/2 μg/ml, similar to those for imipenem and meropenem ($MIC_{50/90}$s, 0.12/1 and 0.12/1 μg/ml, respectively) and two- to 32-fold lower than other comparators. In time-kill experiments against one strain each of *B. fragilis*, *B. thetaiotaomicron*, *P. bivia* and *Clostridium perfringens*, MK0826 was bactericidal (≥ 3 $\log_{10}$ killing after 48 h) at and above the MIC (0.25, 0.5, 0.5, 0.06 μg/ml, respectively).

1.5 Faropenem

The *in vitro* activity of faropenem (Bayer AG, Wuppertal, Germany) (**Figure 1**) was reported by D Felmingham *et al.* (GR Micro Ltd., London, UK), with MIC_{90}s of 0.008, 0.25 and 0.5 μg/ml, respectively, against penicillin-sensitive, -intermediate and -resistant *S. pneumoniae*. MIC_{90}s against haemolytic streptococci were 0.03 - 0.06 μg/ml, against MSSE were 0.12 μg/ml and against MRSA were > 64 μg/ml. Faropenem's MIC_{90} for Gram-negative bacteria ranged from 0.015 to 4 μg/ml, except for *Serratia marcescans* (MIC_{90}, 16 μg/ml), *Stenotrophomonas maltophilia* (MIC_{90}, > 64 μg/ml) and *P. aeruginosa* (MIC_{90}, > 64 μg/ml) (D Felmingham *et al.*).

Faropenem exhibits excellent penicillin-resistant anti-*S. pneumoniae* activity, with MIC_{90}s of 1 μg/ml, as compared with imipenem, penicillin, amoxicillin/

clavulanate, cefprozil, cefuroxime axetil, azithromycin, clarithromycin and trimethoprim/sulphamethoxazole, with MIC_{90}s of 0.5, 8, 4, 16, 8, > 16, > 16 and > 4 μg/ml, respectively (JA Black *et al.*, Creighton Univ. Sch. Med., Omaha, NE). Faropenem exhibited excellent bactericidal activity against common respiratory pathogens including *M. catarrhalis*, *H. influenzae* and *S. pneumoniae* and was more bactericidal than cephalosporins and macrolides against a penicillin-resistant strain *of S. pneumoniae* (JA Herrington *et al.*, Bayer Corp, West Haven, CT), making faropenem a good candidate for treating respiratory tract bacterial infections. Faropenem was reported to be resistant against a panel of penicillinases and cephalosporinases from Gram-positive and Gram-negative bacteria (A Dalhoff *et al.*, Bayer AG Pharma Res. Ctr., Wuppertal, Germany). The MIC values of faropenem against *S. pneumoniae*, *Enterococcus faecalis* and *Escherichia coli* track with the extensive PBP1, PBP2 and PBP3 binding for *S. pneumoniae*, PBP5, PBP3, PBP1 and PBP4 binding for *E. faecalis* and PBP2, PBP4, PBP1 PBP3 and PBP5 binding for *E. coli* (A Dalhoff *et al.*). Morphological studies of faropenem binding affinity for *S. aureus* PBP1/PBP3 and *E. coli* PBP3 confirmed the primary target activity in whole bacteria (A Dalhoff and K Okamoto, Bayer AG).

Pharmacokinetic (PK) profiles of a single 300 mg dose of faropenem daloxate in human subjects provided the following range of values:

- Males: $AUC_{O\rightarrow\infty}$ 26 - 32.8 mg.h/l; C_{max}, 13.5 - 13.8 μg/ml; $t_{½}$, 0.88 - 1.32 h; and time over MIC, 4.6 - 5.5 h
- Females: $AUC_{O\rightarrow\infty}$ 27.6 - 31.6 mg.h/l; C_{max}, 12.9 - 13.3 μg/ml; $t_{½}$, 0.91 - 1.09 h; and time over MIC, 4.5 - 5.1 h (JT Lettieri *et al.*, Bayer Corp., West Haven, CT)
- Escalating dosing of faropenem daloxate from 300 to 1200 mg resulted in approximately linear increases in all PK parameters (AUC, C_{max}, time over MIC), with similar $t_{½}$ (1.14 - 1.39 h) (U Schuehly *et al.*, Bayer AG, Wuppertal, Germany)

2. Quinolones

2.1 Gatifloxacin

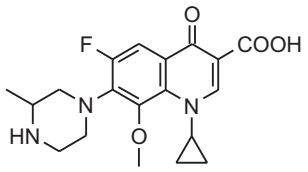
Gatifloxacin (Bristol-Myers Squibb, Princeton, NJ) (**Figure 2**), a recently launched novel, 8-methoxy quinolone, was again highlighted in approximately two dozen posters. RC Owens *et al.* (Maine Med. Center, Portland, ME) presented data on a Monte Carlo analysis of the probability of gatifloxacin, moxifloxacin and levofloxacin achieving the target AUC:MIC ratio of 30. Using *in vitro* susceptibility data, free drug AUC:MIC ratios were calculated (using standard PK numbers reported by each company for their product) and the Monte Carlo analysis indicates a 98.8, 97.9 and 81.1% probability of achieving the AUC:MIC ratio of 30 in man for gatifloxacin, moxifloxacin and levofloxacin, respectively (RC Owens *et al.*). The 'dose-dependent total antimicrobial' effect of gatifloxacin over ciprofloxacin was confirmed by SH Zinner *et al.* (Mount Auburn Hosp., Cambridge, MA) in an *in vitro* pharmacodynamic model of anti-pneumococcal activity. From this model, a 1400 mg dose of ciprofloxacin would be required to attain the same clinical effect of the standard 400 mg gatifloxacin q.d. dosage (SH Zinner *et al.*). Confirmation of gatifloxacin activity was reported by PD Lister and JA Black (Creighton Univ. Sch. Med., Omaha, NE) in an *in vitro* PD model, in which killing by gatifloxacin was compared with levofloxacin. Both gatifloxacin and levofloxacin exhibited similar PK-based killing and lack of emergence of resistant *S. pneumoniae* isolates *in vitro*.

2.2 BMS 284756 (T-3811)

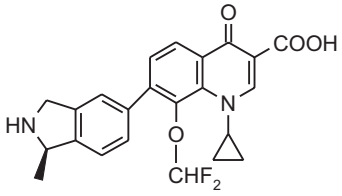
BMS 284756 (T-3811) (**Figure 2**) is a novel gyrase inhibitor without the C-6 fluorine typical of the older generation fluoroquinolones. With a broad spectrum of antibacterial activity, especially against Gram-positive organisms, as well as good anaerobic activity, BMS 284756 may replace trovafloxacin as the anaerobic, broad spectrum quinolone. E Gradelski *et al.* (Bristol-Myers Squibb, Wallingford, CT) presented on the broad spectrum activity of BMS 284756 comparing this new agent with five other quinolones (trovafloxacin, moxifloxacin, levofloxacin, ofloxacin and ciprofloxacin) against over 400 pathogens. BMS 284756 was of equal to or greater activity than all other quinolones except against *Enterococcus faecium*, with MIC_{90}s of 0.03, 2, 0.03, 2, 0.25, 0.25, 0.12, 0.5, 0.5 and 8 μg/ml, respectively, against MSSA, MRSA, MSSE, MRSE, β-haemolytic streptococci, viridans streptococci, ciprofloxacin-susceptible *S. pneumoniae*, ciprofloxacin-resistant *S. pneumoniae*, *E. faecalis* and *E. faecium*. The quinolones could be grouped into 'more active' (BMS 284756, trovafloxacin and moxifloxacin), with MIC_{90}s of BMS 284756 ranging

Figure 2: Chemical structures of quinolones.

Gatifloxacin



BMS 284756 (T-3811)



from equal to, or up to eight-fold more active; whereas the 'less active' quinolones (levofloxacin, ofloxacin and ciprofloxacin) have MIC_{90}s four- to 32-fold less active than BMS 284756 (E Gradelski *et al.*). BMS 284756 had the best activity against ciprofloxacin-resistant *S. pneumoniae*, with an MIC_{90} of 0.5 μg/ml, as compared with trovafloxacin, moxifloxacin, levofloxacin, ofloxacin and ciprofloxacin with MIC_{90}s of 4, 2, 8, 16 and 16 μg/ml, respectively. This activity was independently confirmed in several other *in vitro* studies reported, including the SENTRY data report by AC Gales *et al.* (Univ. Iowa College of Medicine, Iowa City, IA), who reported the same rank order of susceptibility (based on MIC_{90}s) with BMS 284756 > trovafloxacin > gatifloxacin > levofloxacin > ciprofloxacin against all *S. pneumoniae* isolates (regardless of ciprofloxacin susceptibility). Among these 257 *S. pneumoniae* isolates reported from Latin America medical centres, 72.4% were penicillin-susceptible and 13.2% had high-level penicillin resistance. Breakpoint/MIC_{90}s for BMS 284756, trovafloxacin, gatifloxacin, levofloxacin and ciprofloxacin were 16, 4, 2, 2 and ~ 0.5 μg/ml, respectively, providing BMS 284756 with the highest cushion of peak serum drug level to MIC ratio. In a separate SENTRY report, RN Jones *et al.* (Univ. of Iowa) reported on ciprofloxacin-resistant Gram-positive cocci susceptibility to BMS 284756 and two other quinolones (gatifloxacin and trovafloxacin), as well as ampicillin, linezolid and vancomycin. Against 197 ciprofloxacin-resistant *S. pneumoniae* isolates, BMS 284756 was 100% susceptible at or below the estimated breakpoint of 2 μg/ml (or possibly 4 μg/ml), whereas ciprofloxacin was 100% non-susceptible (resistant) and trovafloxacin and gatifloxacin were 94.1% susceptible. Likewise, in a SENTRY report of 24,961 isolates from Europe and the USA from 1999, of which 11.3% of the isolates (955) were *S. pneumoniae*, the MIC_{90}s for BMS 284756, ciprofloxacin, gatifloxacin and trovafloxacin were 0.12, 2, 0.5 and 0.25 μg/ml, respectively (RN Jones *et al.*).

The anti-anaerobic activities of BMS 284756 were reported by DW Hecht and JR Osmolski (Hines VA Hosp., Loyola Univ. Med. Center, Maywood, IL). The anti-anaerobic activity of BMS 284756 was equal to or superior to other quinolones tested (trovafloxacin, moxifloxacin) and superior to typical anti-anaerobic agents such as clindamycin and cefoxitin. With the exception of several anaerobes (i.e., *Fusobacterium mortiforum/varium*, *B. thetaiotaomicron*, *Porphyromonas anaerobius* and *Peptostreptococcus loeschii*), BMS 284756's MIC_{90}s ranged from 0.05 - 2 μg/ml, providing broad spectrum anaerobic coverage, whereas trovafloxacin's MIC_{90}s were generally equal to BMS 284756 and moxifloxacin's MIC_{90}s were generally four-times less active (DW Hecht and JR Osmolski). Imipenem maintained the best overall anti-anaerobic activity with MIC_{90}s ranging from ≤ 0.015 - 0.5 μg/ml; whereas clindamycin (MIC_{90}s ranging from ≤ 0.015 - > 64 μg/ml), piperacillin/tazobactam (MIC_{90}s ranging from < 0.03 - 16 μg/ml) and cefoxitin (MIC_{90}s ranging from 0.125 - 128 μg/ml) continue to show resistance emergence and reduced utility as the 'gold standards' of anaerobic coverage.

An SAR study of BMS 284756 and analogues was presented by P Wu *et al.* (Bristol-Myers Squibb, Wallingford, CT). The specific mechanism of action of this novel quinolone was shown to be a putative DNA gyrase A inhibitor in a demonstration of both DNA gyrase supercoiling inhibition and formation of the cleavable complex (CC_{50}; specific for GyrA inhibitors). Target-based inhibition as measured by the supercoiling inhibition assay ranks activity of BMS 284756 = ciprofloxacin > levofloxacin > moxifloxacin, with IC_{50} values of 0.17, 0.18, 0.32 and 0.36 μg/ml, respectively (P Wu *et al.*). Verification of the GyrA target activity was provided in the CC_{50} assay, in which novobiocin as a negative-control GyrB inhibitor (CC_{50} > 3000 μg/ml), was compared with the four quinolones, whose CC_{50}s ranged from 1.23 - 6.55 μg/ml. Little difference was observed for microbiological activity or target-based inhibition of closely related analogues of BMS 284756, including the

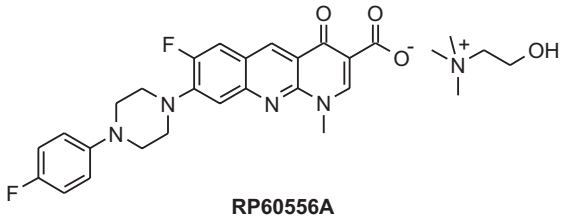
S-isomer, the C-6 fluorine analogue and the -$OCHF_2$ ether-minus analogue, however there were differences in selectivity and cytotoxicity (LE Lawrence *et al.*, Bristol-Myers Squibb; H Yamada *et al.*, Toyama Chem. Co, Toyama, Japan), with BMS 284756 being among the quinolones with the greatest selectivity over the human topoisomerase II inhibition. Microbial physiology macromolecule precursor studies demonstrated BMS 284756 to be the most selective quinolone for targeting DNA synthesis over RNA or protein synthesis, with AUC/IC_{50DNA} ratios of 822, 640, 103 and 44 for BMS 284756, moxifloxacin, levofloxacin and ciprofloxacin, respectively; as well as C_{max}/IC_{50DNA} ratios of 78, 60, 12 and 10, for BMS 284756, moxifloxacin, levofloxacin and ciprofloxacin, respectively, making BMS 284756 the most specific DNA targeting quinolone tested (H Ho *et al.*, Bristol-Myers Squibb).

The bactericidal activity of BMS 284756 was reported by BM Ryan *et al.* (Bristol-Myers Squibb) for inhibition of MSSA and MRSA. Matching human PK/PD parameters to microbiological activity (killing and post-antibiotic effect), Ryan *et al.* demonstrated C_{max}/MIC and AUC/MIC ratios of 188 and 1987, respectively, for BMS 284756 against methicillin-sensitive *S. aureus* (MSSA), whereas levofloxacin was 22.8 and 190, respectively and ciprofloxacin was 5.9 and 27.4, respectively. Likewise for MRSA, the demonstrated C_{max}/MIC and AUC/MIC ratios of 94 and 993, respectively for BMS 284756 against MSSA, whereas levofloxacin was 22.8 and 190, respectively and ciprofloxacin was 11.9 and 54.8, respectively (BM Ryan *et al.*), demonstrating a predicted greater potency *in vivo*. Quinolone-resistant strains of MSSA, either GyrA or GyrA:GrlA, were compared for BMS 284756, ciprofloxacin and levofloxacin susceptibility and BMS 284756 was shown to maintain the same microbiological superiority with both mutants, exhibiting four- to eight-fold and eight- to 16-fold greater activity than levofloxacin and ciprofloxacin, respectively, against the wild type MSSA strain, six- to eight-fold and 12- to 16-fold greater activity against the GyrA mutant for both levofloxacin and ciprofloxacin, respectively and four- and ≥ eight-fold activity over levofloxacin and ciprofloxacin, respectively against the double GyrA:GrlA mutant (L Lawrence *et al.*, Bristol-Myers Squibb). In examining the rate of resistance emergence frequency, site of mutation and cross-resistance between BMS 284756, ciprofloxacin, moxifloxacin and levofloxacin, SL Hartman-Neumann *et al.* (Bristol-Myers Squibb) reported on the selection and characterisation of mutants of *S. pneumoniae*, selected by either ciprofloxacin or BMS 284756. Ciprofloxacin-selected mutants saw MIC values change from 0.5 μg/ml (WT = wild type) to 2 μg/ml in the first step and 16 μg/ml in the second step mutants for ciprofloxacin, 0.5, 2 and 16 μg/ml, respectively for levofloxacin and 0.125, 0.25 and 2 μg/ml for moxifloxacin, respectively, whereas BMS 284756 MIC values were 0.015, 0.06 - 0.25 and 0.5 μg/ml, respectively. This indicates that ciprofloxacin loses susceptibility by the first step mutation, levofloxacin by the second step mutation, moxifloxacin by the second step mutation, but BMS 284756 maintains susceptibility into the second step (and beyond) (SL Hartman-Neumann *et al.*). In the converse experiment, BMS 284756 selected mutants of *S. pneumoniae*, MIC values rose from 0.015 μg/ml (WT) to 0.06 - 0.125 μg/ml (first step) to 0.25 μg/ml (second step) to 2 μg/ml (third step) to 8 - 16 μg/ml (fourth step), whereas ciprofloxacin's MIC values rose from 0.5 μg/ml (WT) to 1 μg/ml (first step) to 16 μg/ml (second step) to 16 μg/ml (third step) to 32 μg/ml (fourth step); levofloxacin's MIC values rose from 0.5 μg/ml (WT) to 1 μg/ml (first step) to 16 μg/ml (second step) to 16 μg/ml (third step) to 32 μg/ml (fourth step); and moxifloxacin's MIC values rose from 0.125 μg/ml (WT) to 0.25 μg/ml (first step) to 4 μg/ml (second step) to 4 - 8 μg/ml (third step), to 64 μg/ml (fourth step). Thus, ciprofloxacin-resistant laboratory mutants with loss of ciprofloxacin susceptibility by the first or second step and cross-resistant to levofloxacin and moxifloxacin by the second step, are covered by BMS 294756, suggesting the possibility of BMS 284756 being used to cover first and second step quinolone-resistant mutants in the clinical setting.

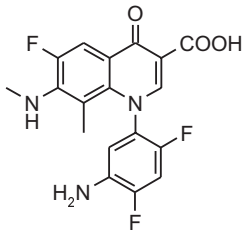
2.3 Non-fluorinated quinolones

Researchers presented a series of posters on their non-fluorinated quinolones (non-FQ) (JL Gray *et al.*, TW Morris *et al.*, S Roychoudhury *et al.*, I Critchley *et al.*, Procter and Gamble). A series of non-FQ compounds were described in a chemistry-based poster. The genesis of the non-fluorinated compounds was the desire to move away from the genotoxicity associated with the presence of the fluorines. It was found that it was possible to synthesise a series of compounds without the C6 fluorine that could be as potent as the fluorinated congeners. An interesting side finding was substitution of a chlorine at C6, along with aminoethylpyrollidone at C7 and O-methoxy at C8 yields a compound of

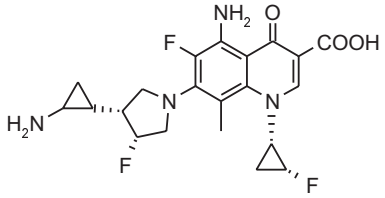
Figure 3: Chemical structures of quinolone-related agents.



RP60556A



WQ-2944



D61-1113

exceptional potency that is equipotent with an identical compound with C6 fluorine. Profiling of the metabolic specificity of these compounds, by monitoring DNA, RNA, protein and cell wall synthesis in *S. aureus*, indicated that the DNA synthesis inhibition was in line with so-called third generation quinolones. In this regard, the DNA synthesis inhibition was more specific than observed in earlier compound generations. In general, the non-FQs are rapidly bactericidal *in vitro* and are claimed to have activity in mouse infection models. A series of *S. aureus* mutants against the non-FQ compounds were selected *in vitro*. These were interesting in that non-FQs selected mutations at sites outside the quinolone resistance determining region (QRDR) where FQ mutations are usually found. 'Non-traditional' mutations in GrlA include His103-Tyr and SeR52-Arg; Glu472-Val in GrlB and Glu477-Val in GyrB. Prior studies had indicated that the common QRDR mutation Ser84-Leu led to only two- to four-fold MIC increases for non-FQ compounds. However, in the presence of these other mutations, particularly the GrlA changes outside the QRDR, MIC increases of 512-fold were observed. Two posters rounded out the section, one on the activities of the non-FQs on clinical isolates from the USA and one on *Mycoplasma*, *Chlamydia* and *Legionella* susceptibilities to these agents. The conclusions of the clinical isolate study were that non-FQs are particularly potent against pneumococci and other streptococci and equipotent against Gram-negatives and obligate anaerobes. The non-FQs also had advantages against ciprofloxacin-resistant staphylococci over the fluorinated quinolones. With regard to the atypical pathogens, the non-FQs compounds were active against *Legionella pneumophila* with activity similar to clarithromycin and levofloxacin. With regard to *Chlamydia pneumoniae*, the compounds were active, with similar potency to clarithromycin and trovafloxacin, but with eight- to 16-fold greater activity than levofloxacin. The non-FQs were of similar activity to levofloxacin and trovafloxacin when tested against *Mycoplasma pneumoniae*. Clarithromycin had activity against these organisms superior to all quinolones, non-fluorinated or otherwise.

3. Quinolone-related agents

3.1 RP60556A

RP60556A (RP) (**Figure 3**), the cholinate salt of a benzo[b]-naphthyridone derivative, is structurally related to quinolones. It is active against aerobic Gram-positive cocci (staphylococci, streptococci and enterococci) and anaerobes, with MIC values ranged from 0.12 - 2 μg/ml (N Berthaud *et al.* Aventis Pharma, Center de Recherche de Vitry-Alfortville, Vitry sur Seine, France). The MIC values against *Enterobacteriae* and *Pseudomonas* were generally ≥ 64 μg/ml. When tested against a panel of quinolone-sensitive and quinolone-resistant *S. aureus* strains (with mutations in GyrA, GrlA and/or NorA), RP60556A demonstrated same level of activity (MIC values from 0.25 - 0.5 μg/ml) (N Berthaud *et al.*, Aventis Pharma). It was also equally active against quinolone-sensitive and quinolone-resistant *S. pneumoniae* clinical isolates, including the strains resistant to quinolone, penicillin, macrolides, lincosamides and streptogramin B. The MIC values ranged from 0.5 - 1 μg/ml. Against *S. aureus* and *S. epidermidis*, RP60556A had strong and quick bactericidal activity, generally occurring at concentrations ranging from 2 - 4 times MIC within a 3 - 6 h period (N Berthaud *et al.*, Aventis Pharma). Against streptococcus Group A, RP60556A was generally bactericidal at concentrations ranging from 2 - 4 times MIC within a 3 h period. The hypothesis of transepidermal diffusion of RP was

supported by the experiment by F-X Bernard *et al.* (Aventis Pharma). These results suggest the potential of RP as a topical anti-Gram-positive agent.

3.2 D61-1113

D61-1113 (**Figure 3**) is a leading compound from a series of 5-amino-1-[2-(*S*)-fluoro-1-(*R*)-cyclopropyl]-8-methylquinolones with the modified 3-(aminomethyl)pyrrolidin-1-yl substituents at the C7-position. It has the 3,4-*cis*-oriented (3S, 4R)-4-(1-aminocyclopropyl)-3-fluoropyrrolidin-1-yl C7 substituent. Its antibacterial activity, pharmacokinetics and preliminary safety profiles were described by H Takahashi *et al.* (Daiichi Pharmaceutical Co. Ltd., Japan). D61-1113 showed a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria. It was more potent than reference quinolones, vancomycin, teicoplanin and linezolid especially against clinical isolates of Gram-positive bacteria including ofloxacin-resistant MRSA, MRCNS, PRSP and VRE. The activity of D61-1113 against clinical isolates of Gram-negative bacteria was comparable with those of aforementioned reference drugs. It achieved high C_{5min} in the serum and good tissue distribution pattern in liver, kidney and lung ($AUC_{tissue}/AUC_{serum} > 3.7$) after iv. administration to experimental animals. The serum protein binding of D61-1113 in human was 75%. D61-1113 also showed favourable safety profiles. It did not prolong QT interval in monkeys under the repeated-dose toxicity experimental conditions. D61-1113 was selected for further evaluation as the promising candidate for the treatment of serious infections due to Gram-positive bacteria.

3.3 WQ-2944/WQ-3402

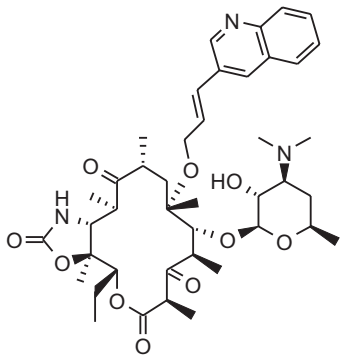
The SAR of novel acidic, 7-amino or 7-alkylamino-1-(5-amino-2,4-difluorophenyl)-8-methylquinolones was studied by Y Kuramoto *et al.* (Wakunaga Pharmaceutical Co. Ltd., Hiroshima, Japan). The alkyl chain on the amino group at the 7-position is essential to enhance the antibacterial activity against Gram-positive resistant strains. Although not affecting the activity against Gram-positive bacteria, the increase in the alkyl chain length reduced the activity against Gram-negative bacteria. WQ-2944 (**Figure 3**), with methylamino group at the 7-position, was the most potent acidic quinolone tested against clinical isolates including quinolone-resistant strains. The therapeutic effects of WQ-3402 (ethanolamine salt of WQ-2944) were superior to those of levofloxacin. WQ-2944 appears to be a new antibacterial agent with potent activity, especially against quinolone-resistant Gram-positive bacteria.

4. Ketolides

4.1 Telithromycin

Aventis researchers presented several posters on their new ketolide, telithromycin, specifically developed for respiratory tract infections. A poster presented by B Leroy *et al.* (Aventis, Bridgewater, NJ and Aventis Pharma, Hoechst Marion Roussell Romainville, Romainville, France) reported the Phase III results of the efficacy of telithromycin in treating bacteraemia with community-acquired pneumonia (CAP). The clinical trials included 755 patients ranging in age from 18 - 65. A dose of 800 mg once daily for 7 - 10 days was evaluated in three multicentre, randomised, double-blind comparator studies (comparators: amoxicillin 100 mg three times a day for ten days, clarithromycin 500 mg twice daily for ten days and trovafloxacin 100 mg once daily for 7 - 10 days). Clinical and bacteriological assessments were made throughout the course of the study. The clinical and bacteriological efficacy of telithromycin post-therapy and test of cure (TOC) were excellent. The clinical efficacy was 91.8% and bacteriological outcome was 90.0%. The clinical success rate for telithromycin for bacteraemia was 90% (27/30) and 88.5% (23/26) for documented pneumococcal bacteraemia. Telithromycin at 800 mg once daily for 7 - 10 days was as effective as the comparators in the treatment of CAP. B Leroy *et al.* (Aventis and Aventis Pharma, Hoechst Marion Roussell Romainville) presented on the *in vitro* susceptibility of telithromycin against clinical isolates of respiratory pathogens. This study obtained clinical isolates from adult patients with community-acquired respiratory tract infections (RTI) from a multicentre clinical trial. The key respiratory tract pathogens for this study were identified as *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *M. catarrhalis*, *S. aureus* and *S. pyogenes*. Patients were treated with an oral dose of 800 mg of telithromycin once daily: 7 - 10 days for CAP, five or ten days for acute maxillary sinusitis (AMS) and five days for acute exacerbation of chronic bronchitis (ACEB) or tonsilopharyngitis. A single dose of 800 mg of telithromycin provided excellent coverage for clinical isolates of key respiratory tract pathogens. The clinical efficacy and bacteriological cure were evaluated at

Figure 4: Chemical structure of ABT-773.



post-therapy/TOC at days 17 - 21, except for tonsilio-pharyngitis evaluated at days 16 - 20. The clinical and bacterial cure rates for telithromycin against the key pathogens were: *S. pneumoniae* (94.6%), *H. influenzae* (79.3%), *H. parainfluenzae* (85.7%), *M. catarrhalis* (92.3%), *S. aureus* (100.0%) and *Streptococcus pyogenes* (88.3%).

4.2 ABT-773

ABT-773 (**Figure 4**) is a 14-membered ketolide currently under clinical development by Abbott Laboratories (Abbott Park, IL) for treatment of respiratory infections. It was reported to be active against β-lactam and macrolide-resistant streptococci, *H. influenzae* and *M. catarrhalis*. ABT-773 was reported to be bactericidal against macrolide-sensitive and resistant *S. pneumoniae* with faster time kill kinetics than erythromycin (N Ramer *et al.* Abbott Labs.). At 8 times MIC, 2 h drug exposure, the *in vitro* post-antibiotic effect (PAE) for ABT-773 was 2 - 4 h and 5 - 6 h for macrolide resistant ($MIC_{ABT-773}$ 0.004 - 0.008 μg/ml and $MIC_{erythromycin}$ > 8 μg/ml) and sensitive ($MIC_{ABT-773}$ 0.004 - 0.008 μg/ml and $MIC_{erythromycin}$ 0.008 - 0.12 μg/ml) strains, respectively. A 4 - 7 h *in vitro* PAE was shown for *H. influenzae* (MIC 1 - 2 μg/ml) and *M. catarrhalis* (MIC 0.015 - 0.06 μg/ml). GA Pankuch *et al.* (Hershey Medical Center, Hershey, PA) also reported similar results. Dosing at 6 mg/kg resulted in *in vivo* PAE (murine thigh infection) of 11 h with *S. pneumoniae* and of 5 - 9 h with *S. aureus* (DR Andes *et al.*, U Wisconsin, Madison, WI). The 24 h AUC/MIC best predicted the *in vivo* activity of ABT-773 for both *S. pneumoniae* and *S. aureus* infections. The protein binding was 90%. ABT-773 was active against fluoroquinolone-resistant (*parC* and/or *gyrA* mutants) *S. pneumoniae* (L Almer *et al.*, Abbott Labs.) both *in vitro* (MIC_{90} = 0.03 μg/ml) and *in vivo* in neutropoenic rat thigh infection model (ED_{50} for a 2 log reduction in bacterial burden of 2.3 mg/kg/day). The agent was bactericidal at 24 h to all pathogens tested (LM Edine *et al.* and KL Credito *et al.*, Hershey Medical Center, Hershey, PA).

ABT-773 also showed both *in vitro* (MIC_{90} = 0.03 - 0.06 μg/ml) and cellular (HL-60 cells) activity against *L. pneumophila* (K Sens *et al.*, VA Medical Center, Pittsburgh, PA). Susceptibility testing of clinical isolates of *S. pneumoniae* obtained from upper respiratory tract of children carrying erythromycin-resistant genes (including erythromycin MIC > 256 μg/ml, strains), by C Johnson *et al.* (Univ. Alabama, Birmingham, AL), showed minimal increase of the MIC values of ABT-773 (MIC_{90} of 0.125, 0.032 and 0.5 μg/ml for mefE, ermB and both genes, respectively). These isolates remained susceptible to ABT-773, which was reported to have similar potency as the newer quinolones and is two- to three-times more active than telithromycin (Aventis). Posters by Canadian researchers (BM Raily *et al.*, Univ. Toronto and K Weiss *et al.*, Univ. Montreal, Montreal, Canada) reported similar activities for ABT-773 against streptococci with one exception. A *S. pneumoniae* strain with a MIC of 128 μg/ml was identified, raising concern of existing resistance in the community. In Japan, surveillance data reported similar potency of ABT-773 for all *S. pneumoniae*, MSSA, *S. pyogenes* tested and also observed one quinolone-resistant MSSA showing MIC of ABT-773 at > 128 μg/ml (T Fujikawa *et al.*, Toho Univ., Tokyo, Japan).

In a dose-escalation study (RS Pradhan *et al.*, Abbott Labs.) ranging from 100 - 1200 mg in fasting human subjects, AUC deviated from linearity primarily in the 100 - 400 mg dose range and once daily dosing of this agent was suggested. Higher accumulation than the dose proportion was observed in this range. The C_{max} increased proportionally with dose and mean $t_{½}$ ranged between 3.6 and 6.6 h. The bioavailability of ABT-773 is unaffected by food (RS Pradhan *et al.*, Abbott Labs.).

4.3 CP-654743

CP-654743, a fluoro ketolide, targeting respiratory tract infections was reported (T Kaneko *et al.*, Pfizer, Inc., Groton, CT) with MIC values against key pathogens (**Table 1**).

In murine peritonitis and pulmonary infection models (D Girard *et al.*, Pfizer, Inc., Groton, CT) by MLS-phenotype, penicillin-resistant *S. pneumoniae*,

Table 1: MIC values of CP-654743 against key pathogens.

Pathogen	*S. pneumoniae*	*S. pneumoniae ermB*	*S. pneumoniae ermA*	*H. influenzae*	*S. pyogenes ermB/ermA*	*S. pyogenes mefA*
MIC	0.004 μg/ml	0.125 μg/ml	0.25 μg/ml	2.0 μg/ml	6.3 μg/ml	1.0 μg/ml

the mean oral ED_{50} values were 22.7 and 28 mg/kg, respectively. In gerbil middle ear *H. influenzae* infection model, the mean ED_{50} was 23 mg/kg.

5. New β-lactams/cephalosporin

5.1 Cefditoren

Cefditoren, formerly ME-1206, is a novel oral cephalosporin that has potent activity against the bacterial species most commonly associated with respiratory tract infection. A number of posters investigated the *in vitro* activities of cefditoren against clinical isolates of *H. influenzae, M. catarrhalis, S. pneumoniae* and non-pneumococcal streptococci. DF Sahm *et al.* (MRL, Herndon, VA and Brentwood, TN) tested the activities of cefditoren against 1372 *H. influenzae* strains (35.2% β-lactamase-positive) and 843 *M. catarrhalis* strains (83.7% β-lactamase-positive) collected from US hospitals during 1999 - 2000. Also tested were other antimicrobials such as cefuroxime, cefprozil, ceftriaxone, amoxicillin/clavulanate, ampicillin, erythromycin, clarithromycin, azithromycin, trimethoprim/sulphamethoxazole (SXT) and levofloxacin. Cefditoren has excellent activity against *H. influenzae* (MIC values ranging from ≤ 0.008 to 0.25 μg/ml); in contrast, the MIC values of comparator β-lactams ranged from ≤ 0.015 to 32 μg/ml. β-lactamase production in *H. influenzae* did not affect the MIC distribution for cefditoren. The cefditoren MIC_{90} was 0.015 μg/ml for both β-lactamase-positive and β-lactamase-negative isolates, equivalent to levofloxacin, similar to ceftriaxone and more active than amoxicillin/clavulanate. Based on MIC_{90} values the hierarchy of activity of tested β-lactams for all *M. catarrhalis* isolates was as follows: amoxicillin/clavulanate (0.25 μg/ml) > cefditoren = ceftriaxone (0.5 μg/ml) > cefuroxime (2 μg/ml) > cefprozil (4 μg/ml) > ampicillin (8 μg/ml). The MIC_{90}s for cefditoren were higher against β-lactamase-positive (0.5 μg/ml) than β-lactamase-negative (0.015 μg/ml) isolates. The MIC_{90}s for macrolides (0.03 - 0.25 μg/ml) were lower than cefditoren.

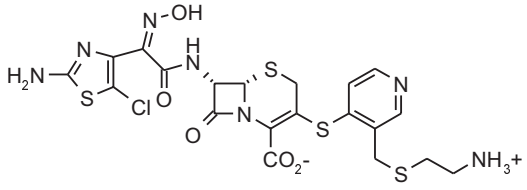
A similar study was conducted by C Thornsberry *et al.* (MRL) against 2597 *S. pneumoniae* isolates (62.9% penicillin-susceptible, 20.0% penicillin-intermediate and 17.1% penicillin-resistant) collected in 2000. Based on MIC_{90}s, the order of activity for all *S. pneumoniae* isolates was cefditoren (0.5 μg/ml) > ceftriaxone = levofloxacin (1 μg/ml) > penicillin = amoxicillin/clavulanate (2 μg/ml) > ampicillin = cefuroxime (4 μg/ml) > azithromycin = SXT (> 4 μg/ml) > cefprozil (8 μg/ml). Cefditoren had higher MIC_{90}s against penicillin-resistant strains (PEN-R, 1 μg/ml) than penicillin-susceptible strains (PEN-S, 0.03 μg/ml), yet it was more active against PEN-S, PEN-I (penicillin-intermediate) and PEN-R isolates than other β-lactams tested. Cefditoren was also active against strains resistant to other cephalosporins and levofloxacin. Time-kill experiments were performed by D Draghi *et al.* (MRL) against nine recent clinical isolates of *S. pneumoniae* with different phenotypes. Results showed that cefditoren had potent bactericidal activity against strains of *S. pneumoniae* that were not only resistant to penicillin, but also other antimicrobial classes including fluoroquinolones, macrolides and SXT.

The activities of cefditoren against non-pneumococcal streptococci were evaluated by DF Sahm *et al.* (MRL). Isolates (n = 450) of viridans streptococci, 917 *S. pyogenes* and 800 other β-haemolytic streptococci were tested. Based on MIC_{90}s, the order of activity for viridans streptococci isolates was cefditoren (0.5 μg/ml) > penicillin (2 μg/ml) > amoxicillin/clavulanate = ampicillin = cefuroxime (4 μg/ml) > cefprozil (16 μg/ml). The MIC_{90}s for cefditoren against *S. pyogenes* and other β-haemolytic streptococci were 0.015 and 0.06 μg/ml, respectively. Cefditoren was the most active β-lactam tested. All this data demonstrated cefditoren's potential as a treatment for respiratory tract infections.

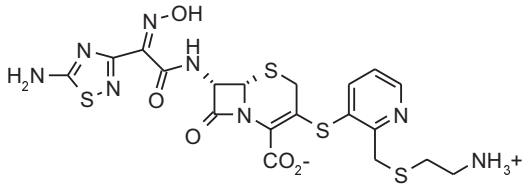
5.2 RWJ-54428 (MC 02479)

RWJ-54428 (MC 02479) (**Figure 5**), a novel cephalosporin, was previously shown to have excellent activity against Gram-positive organisms. The anaerobic activity of this compound was evaluated against 363 anaerobes isolated from clinical specimens by LM Kelly *et al.* (Hershey Med. Ctr, Hershey, PA and Case Western Reserve Univ.,

Figure 5: Chemical structures of new β-lactams/cephalosporins.



RWJ-54428 (MC 02479)



RWJ-333441 (MC 04546)

Cleveland, OH). The results showed it had low MIC values only against β-lactamase-negative anaerobic strains. The investigators suggested that if this compound is to have additional activity against β-lactamase-positive organisms, combination with a β-lactamase inhibitor will be necessary.

5.3 RWJ-333441

Researchers from Microcide Pharmaceuticals, Inc. (Mountain View, CA) presented a series of posters on RWJ-333441 (MC 04546), a new cephalosporin active against a variety of clinically important Gram-positive bacteria, including MRSA. It is a lead compound from a series of 3-(heteroarylthio)cephems. The discovery of this compound was reported by T Glinka *et al.* (Microcide). It has been found that in order to achieve high *in vitro* activity against MRSA it is necessary to append electron-withdrawing substituents at C-3 and C-7 of the cephem core, which increase the reactivity of the β-lactam ring. Optimisation of anti-MRSA activity *versus* stability toward serum-mediated degradation required a fine balance of substituent effect; RWJ-333441 was the one with the superior balance. It displays potent anti-MRSA activity (MIC_{90}, 2 μg/ml), high rat serum stability (6% decomposition over 60 min) and improved pharmacokinetics in rat (total clearance 0.39 l/h/kg). The potency of RWJ-333441 against MRSA was shown to be related to its enhanced affinity for PBP2a by J Blais *et al.* (Microcide). It had high activity against MRSA COL (MIC, 1μg/ml) and high affinity for PBP2a (IC_{50} = 0.8 μg/ml). It was stable to staphylococcal β-lactamases and its rates of hydrolysis by various β-lactamases were comparable with those of cefazolin. The frequency of isolation of resistance to RWJ-333441 in MRSA COL was very low (< 10^{-10} at 2x MIC). Besides excellent anti-MRSA activity, RWJ-333441 was shown to be potent against penicillin-resistant pneumococci, viridans group streptococci and vancomycin-resistant *E. faecalis* (J Blais *et al.*, Microcide). Its MIC_{90} values (2 μg/ml) were similar to vancomycin against MRSA and MRCNS (coagulase-negative staphylococci) and it was active against MRSA with reduced susceptibility to glycopeptides (MIC values 0.125 - 2 μg/ml). RWJ-333441 (MIC_{90}, 1 μg/ml) was more active than ampicillin (MIC_{90}, 2 μg/ml) against vancomycin-, gentamicin- and ciprofloxacin-resistant *Enterococcus faecalis* and it was the most potent agent tested against ampicillin susceptible *E. faecium* (MIC_{90}, 4 μg/ml). RWJ-333441 showed good activity against *H. influenzae* and *M. catarrhalis* (MIC_{90}, 2 μg/ml), but poor activity against other Gram-negative bacteria (MIC_{90} > 128 μg/ml). It demonstrated excellent bactericidal and significant post-antibiotic effects against multi-resistant staphylococci (C Park *et al.*, Microcide). The aqueous solubility of RWJ-333441 at physiological pH (7) is 4.4 mg/ml, which is lower than desired for iv. administration. A prodrug approach was reported by SJ Hecker *et al.* (Microcide) to improve its solubility. Several acyl derivatives of the C(3) primary amino group of RWJ-333441 were prepared and their aqueous solubility, cleavage *in vitro* in serum and conversion to parent drug *in vivo* were measured. The aspartate derivative (RWJ-333442) demonstrated desirable properties and appeared to be suitable for parenteral administration. The activity of RWJ-333441 (administrated as either the active or the prodrug form RWJ-333442) in the mouse sepsis and the neutropoenic mouse thigh models was investigated by D Griffith *et al.* (Microcide). Both forms were shown to be efficacious against MSSA, MRSA and GISA (glycopeptide-intermediate *S. aureus*). The *in vivo* efficacy and potency was similar or superior to current treatment (e.g., vancomycin and Synercid™) for MRSA.

6. β-Lactamases and inhibitors

6.1 ESBLs (extended spectrum β-lactamases)

Reports covering β-lactamase (BLA)-mediated bacterial resistance to β-lactams, especially the third generation cephalosporins, were well represented at the 2000 ICAAC. The patterns of resistance prevalence are both dynamic and complex as well as regional and

Table 2: New ESBLs and AmpC-like BLAs or new bacterial hosts for known BLAs as reported at the meeting.

Organism	Type	BLA name	Residing where?	Note
P. mirabilis	ESBL	TEM-67 (IRT)	Plasmid	T Naas *et al.*, Hospital de Bicetre, le Kremlin-Bicetre, France
P. mirabilis	ESBL	TEM-87	Plasmid	M Perilli *et al.*, U L'aquila, Italy. Tazobactam, IC_{50} 55 nM, Clavulanate, IC_{50} 130 nM
P. mirabilis	AmpC-like	ACC-1	Plasmid	A Ben Assen *et al.*, Charles Nicolle Hospital, Tunis, Tunisia
E. cloacae	ESBL	TEM-80	Plasmid	C Aprin *et al.*, Univ. Bordeaux 2, Bordeaux, France First IRT reported in *E. cloacae*
E. coli		CTX-M-10		A Oliver *et al.*, Hosp. Ramony Cajal, Madrid, Spain
K. pneumoniae	AmpC-like	FOX-5	Plasmid	AM Queenan *et al.*, R.W. Johnson Res. Inst. Raritan, NJ, USA
Enterobacteriaceae	ESBL	Toho-3	70-kb plasmid	Y Ishii, Toho Univ., Tokyo, Japan
Norcardia asteroides	A	AST-1	Chromosome	F Laurent *et al.*, Hospoces Civils de Lyon, Pierre Benite, France
Ochrobactrum anthropi	AmpC-like		Chromosome	CS Higgins *et al.*, Univ. Bristol, Bristol, UK Inducible, inhibited by BRL42715, not by clavulanate
Chryseobacterium indologenes	MBL	IND-1, -2, -2a, -2b, -3, -3a & -4		S Bellais *et al.*, Hospital de Bicetre. Asist. Publ. Paris, France Shared 77 - 90% amino acid identity
Aeromonas sobria	MBL	ImiS		RM Mosi *et al.*, AnorMED, Inc., BC, Canada

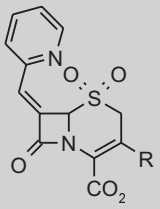
hospital sites dependent. Among the four Ambler-typed BLAs, expression of ESBLs attributed to the most frequently identified mechanism of β-lactam (BL) resistance. *Enterobacteriaceae* such as *E. coli* and *Klebsiella pneumoniae* are the mostly commonly detected TEM and sulfhydryl variable (SHV) classes ESBLs carrying pathogens. New enzymes with distinct substrate profile and mutations continue to evolve. As of July 2000, the TEM variant BLAs reported were up to TEM-90 (many are clavulanate-resistant) and the SHV variants were up to SHV-26. A website monitoring the emergence of new ESBLs set up by G Jacoby of the Lahey Clinic and K Bush of the RW Johnson Pharmaceutical Research Institute can be reached at http://www.lahey.org/studies/webt.htm. Other pathogens harbouring ESBLs include *Proteus mirabilis, Enterobacter cloacae, Morganella morganii, Enterobacter aerogenes* and *Klebsiella oxytoca*.

6.2 SENTRY

SENTRY worldwide surveillance databases from 1997 - 99 indicated the most prevailing Gram-negative bacteria in ICU settings are *Eschericha coli* (9%) = *Klebsiella* (9%) > *Enterobacter spp.* (7%) (D Mathai *et al.*, Univ. Iowa, Iowa City, IA). Characterisation of ESBL phenotype in Europe (EU), North and Latin America and Western Pacific indicated the high prevalence of ESBL in *K. pneumoniae*: Latin America (45%) > Western Pacific (24%) > EU (21%) > US (7%) > Canada (4%). *E. coli* isolates showed similar order, varying at 1 - 8%. Resistance due to ESBL continues to evolve especially in EU and the Western Pacific region. Significant inter-strain and -species dissemination was documented, but the rates of ESBL in US, Latin America and Canada appears stable. RA Bonomo (Veteran's Affairs Medical Center, Cleveland OH) also evaluated ESBLs prevalence in *K. pneumoniae* worldwide and reported that 58.9% of isolates tested positive with *TEM* or *SHV* genes by PCR. Among them, greater than 78% of strains harboured multiple ESBLs that are located on plasmids.

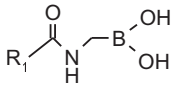
RA Bonomo's group (Veteran's Affairs Medical Center, Cleveland, OH) conducted site-saturation mutagenesis at the conserved S130 and D104-positions of the SHV BLA. Except for the S130G variant, all other *E. coli* variants containing individual

Table 3: The identification of β-lactamase inhibitors.



Compound, R	Enzyme (class) & IC_{50}, μM			
	P99 (C)	TEM (A)	PC1 (A)	GC1 (C)
Tazobactam	49.8	0.32	2.8	3.4
CH_2-O-$COCH_3$	0.5	0.3	2.6	NT
CH = $CHCONH_2$	0.26	0.09	0.1	0.01
CH = CHCN	0.01	0.014	0.72	0.012
CH = $CHCO_2Me$	0.2	0.02	0.3	0.3
CH = $CHClCO_2Me$	0.9	0.07	1.4	0.18
CH = CH-CH = CH	24	68	75	NT
CH = $CHCO_2But$	1.48	NT	240	NT

Figure 6: Structure of acylglycine boronic acid inhibitors.



mutations at S130 were ampicillin and piperacillin-susceptible (MIC < 8 μg/ml). This resistance is limited to clavulanate only, not to sulbactam or tazobactam. Except for D104C, mutations at D104 retained resistance to ampicillin and piperacillin. D104H, 104R and 104K variants showed increased resistance to ceftazidime, as compared with SHV-1, suggesting the positive charge interfering with ceftazidime binding.

6.3 AmpC-type β-lactamases (class C)

Testing and reporting of class C serine BLAs are not standardised as those of ESBLs. These AmpC-like enzymes are cephalosporinases that are not susceptible to clavulanate inhibition and have pIs ranging from 7 - 9. Although AmpC was initially identified as chromosomally located and exhibited inducible phenotype, more and more AmpC-like BLAs are detected at plasmid locations and hyper-express constitutively. A significant number of pathogens (*E. cloacae, K. pneumoniae, Citrobacter fruendii*, etc.) are now known to harbour multiple BLAs belonging to different classes (A & C, A & B, etc.). Specific identification of AmpC-like BLAs and ESBLs in these multiple BLAs-expressing organisms required more complicated protocols and separate confirmation at biochemical levels.

New ESBLs and AmpC-like BLAs or new bacterial hosts for known BLAs were reported (**Table 2**).

By applying electrospray ionisation/mass spectrometry (ESI/MS) analysis to the tazobactam-treated CMY-2 enzyme, RA Bonomo's group (Veteran's Affairs Medical Center, Cleveland, OH) demonstrated the covalent modification of SeR64 of CMY-2 (a class C BLA) by a fragment of tazobactam. No cross-linking to other amino acid residue was observed.

JD Buynak (Southern Methodist Univ., Dallas, TX) and JR Knox (Univ. Connecticut, Storrs, CT) reported a crystal structure of *E. cloacae* GC1 class C β-lactamase in complex with a 7-alkylidene-cephalosporin sulphone inhibitor. Their goal was to identify inhibitors with coverage over as many classes of β-lactamase as possible (**Table 3**).

RA Powers *et al.* (North Western Univ., Chicago, IL) reported the determination of β-lactamase inhibitory activity of eight acylglycine boronic acid inhibitors with the acyl group representing the R1 substitutions of penicillin G, penicillin V, penethicillin, cloxacillin, nafcillin, cephalothin, cephapirin and ceftazidime (**Figure 6**). The K_i values of these inhibitors for AmpC ranged between 0.02 - 0.7 μM, while R1 = CH_3 gave a K_i of 19 μM. The binding affinity of these inhibitors to TEM-1 enzyme was significantly lower. Complexed AmpC crystal structures with two acylglycine boronic acid inhibitors (R1 = cloxacillin or cephalothin) were determined. The inhibitors bound at the region where the R1 side chain of β-lactam typically binds. H-bonding and hydroxyl binding sites were identified. The key interacting residues are either conserved or have equivalent counter part among classes A and C enzymes. The information provides a map of binding sites for future inhibitor design. Inhibitors carrying the R1 chain of nafcillin (K_i = 0.03 μM) and ceftazidime (K_i = 0.02 μM) were shown to potentiate the antibacterial activity of ceftazidime against AmpC expressing *E. cloacae* in disk diffusion plate assay. The ceftazidime side chain containing inhibitor was stated to be at least 6000-fold more selective for AmpC than chymotripsin, trypsin and elastase.

D Tondi (Univ. Modena and Reggio Emilia, Modena, Italy) presented a poster on carboxamide- and sulphonamide boronic acid inhibitors of AmpC BLA. One of the most potent compounds has a K_i of 83 nM

Figure 7: One of the most potent carboxamide- and sulphonamide boronic acid inhibitors of AmpC BLA (K_i = 83nm).

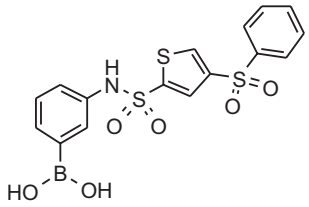
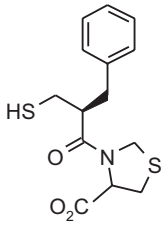


Figure 8: Chemical structure of a thiazolidine and proline mercaptocarboxylate MBL inhibitor.



(**Figure 7**). The crystal structure of AmpC in complex with the inhibitor was determined to 1.9 Å resolution. The inhibitors show little intrinsic antibacterial activity alone. Reversal of amoxicillin resistance by this inhibitor was observed in Gram-positive bacteria only, but not in Gram-negative organisms.

A Patera (North Western Univ., Chicago, IL), in collaboration with Eli Lilly, reported complexed crystal structures of loracarbef (substrate) and cloxacillin (inhibitor) bound to deacylation-deficient AmpC mutant (Q120L) and inhibitor moxalactam bound to WT AmpC. Data obtained is consistent with a β-phase catalytic water attack and both TyR150 and the substrate ring nitrogen stabilised the hydrolytic transition state while inhibitors can act by blocking the activation.

6.4 Metallo β-lactamases (MBL, class B)

CS Higgins (Univ. Bristol, Bristol, UK), in association with SmithKline Beecham, reported a study that showed the N-terminal 20 amino acid residue was not essential for the tetramer formation of L1 MBL from *S. maltophilia*, but significantly reduces the binding affinity of substrates with large aromatic side chains.

F Sanschagrin (Univ. Laval, Ste-Foy, PQ, Canada) presented a poster on using the phage display libraries to facilitate the identification of novel MBL inhibitors. The L1 MBL from *S. maltophilia* was cloned, overexpressed and purified. The purified His-tagged L1 was used to screen phage peptide libraries. Peptides containing consensus sequences were synthesised and tested as MBL inhibitors using nitrocefin as substrate. One of the Cys-7mers-Cys identified showed mixed inhibition with K_i of 10 μM, K_m of 15 μM and K_{cat} of 24 s^{-1}. Addition of Zn^{+2} was able to rescue the peptide-mediated inhibition. Binding of the peptide inhibitor resulted in no detectable structural change of L1 by circular dichroism.

M Gilpin (SKB, Harlow, UK) reported on the SAR of thiazolidine and proline mercaptocarboxylate MBL inhibitors. Activities against four MBLs (L-1, IMP-1, CfiA and Bc II) were evaluated. IC_{50} values of ~ 0.2 - 0.4 μM were achieved with the compound shown in **Figure 8**. The D-stereochemistry at the C-terminal amino acid conferred maximal inhibition of BcII and CfiA while L-1 and IMP-1 were less discriminating. ACE inhibition (captopril) was optimised on the L-series compounds, indicating the selective differences that can be built in for MBL inhibitors.

JL Huber (Merck Research Laboratory, Rahway, NJ) presented a poster on succinic acid derivatives as IMP-1 MBL inhibitors (**Figure 9**).

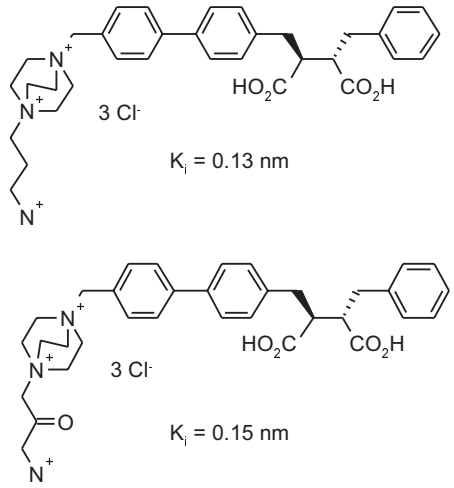
These compounds have no intrinsic antibacterial activity (MIC > 200 μM). A majority of the 18 imipenem-resistant strains of *Pseudomonas* and *S. marcescens* (MIC values 8 - > 256 μg/ml, expressing IMP-1 MBL) tested showed decreased MIC values of imipenem to < 8 μg/ml when used in combination with 12.5 μM of either inhibitors.

T Walsh (Univ. Bristol, Bristol, UK) presented bulgecin A as a weak L1-MBL inhibitor (IC_{50} = 150 μM). This group of compounds has antibacterial and anticancer activities. A patent has been filed for the use of these compounds in pharmaceutical compositions.

7. Streptogramins

Streptogramins belong to an antibiotic class consisting of two structurally unrelated components, group A and group B. The group A compounds are polyunsaturated macrolactones and the group B compounds are cyclic hexadepsipeptides. The group A compounds interfere with the elongation of the peptide chain by preventing the binding of aminoacyl-tRNA to ribosome. The group B compounds stimulate the dissociation of peptidyl-tRNA and may also interfere with the release of the completed peptide. Combination of group A and B compounds works synergistically in inhibiting protein

Figure 9: Chemical structures of some succinic acid derivatives as IMP-1 MBL inhibitors.



synthesis and are strongly bacteristatic, sometimes bactericidal. These antibiotics are produced naturally, but with very poor water solubility. Semisynthetic analogues of these two groups of compounds have been shown to possess improved solubility, enhancing their therapeutic values.

7.1 Synercid™

Synercid™ (quinupristin/dalfopristin; Aventis, Vitry-sur-seine, France), an iv.-only agent, is the first commercially available human antibiotic of the streptogramin class. It was approved for the treatment of serious or life-threatening infections associated with vancomycin-resistant *E. faecium* (but not *E. faecalis*) bacteraemia and for complicated skin and skin structure infections caused by MSSA and *S. pyogenes*. Synercid™ is bacteriostatic against *E. faecium* and bactericidal against strains of methicillin-susceptible and methicillin-resistant staphylococci. The mode of action differs from that of other classes of antibacterial agents such as β-lactams, aminoglycosides, glycopeptides, quinolones, macrolides, lincosamides and tetracyclines. There was no cross-resistance between Synercid™ and these agents.

7.2 RPR-20868/RPR-132552

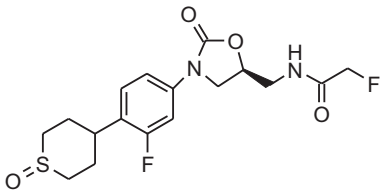
Reported by J-C Barriere (Aventis, Vitry-sur-seine, France) and several Aventis poster presentations (N Berthaud *et al.*, Aventis, Vitry-sur-seine, France), in clinical development by Aventis was the oral streptogramin RPR-20868/RPR-132552, a 30/70 mixture, targeting community acquired respiratory tract infections. The *in vitro* spectrum of bacteriostatic activity included multi-drug resistant Gram-positive cocci (MIC, 0.12 - 0.5 μg/ml) and some Gram-negative bacteria (*H. influenzae* MIC, 1 μg/ml; not active against enterobacteria) responsible for respiratory tract infections. After a 3 h treatment at 8 times MIC (1 - 4 μg/ml), the agent caused a 2.16 - 1.24 $\log_{10}$ CFU/ml drop of ingested *S. aureus* (MSSA and MRSA-MLS_B-CR) inside murine macrophage cells. In a mouse pneumonia model (*S. pneumoniae* MLS_B-resistant), dosing at 120 mg/kg, po. RPR-20868/RPR-132552 resulted in a drop of bacterial count of 2.93 $\log_{10}$ CFU/ml at 6 h post initial treatment. In mouse lung, RPR-20868 accumulated faster than RPR-132552 and was not in accordance with the initial 30/70 ratio administered. RPR-20868/RPR-132552 demonstrated activities in MRSA (MLS_B-resistant) sepsis model with ED_{50} of 44 mg/kg (20 mg/kg for MSSA infection) and in the mouse abscess model with ED_{50} of 60 mg/kg. Clarithromycin was not active in these models at a concentration of 300 mg/kg. When infected with MLS_B *S. pneumoniae*, the mouse sepsis model gave ED_{50} values ranging 24 - 55 mg/kg and the mouse pneumonia model with an ED_{50} at 50 mg/kg.

8. Novel oxazolidinones

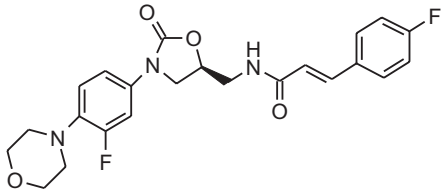
Versicor is participating in a collaboration with Pharmacia & Upjohn to identify, through combinatorial methodologies, and develop a second-generation oxazolidinone with an improved spectrum of activity and improved potency. Versicor (Z Nie *et al.*, Versicor, Fremont, CA) has synthesised VRC-322 (**Figure 10**), a structurally novel C-5 cinnamide, that has an activity profile against Gram-positive pathogens very similar to that of linezolid. The evaluation of a C-5 linezolid analogue library showed an unexpected tolerance for extended C-5 lipophilic substitutions. The investigators hypothesised that there may be an as yet unexplored lipophilic pocket at the ribosomal binding site, which may offer even greater opportunity for second-generation oxazolidinones. The four substituents at the 3-aryl site were also explored by Versicor.

The novel 3′-fluoro-4-thioether phenyloxazolidinones had good activity against Gram-positive pathogens (GW Luehr *et al.*, Versicor, Fremont, CA). VRC-3406, the most active compound in this series, had MIC values of 2, 2 and 1 μg/ml against *S. aureus*, VRE and *S. pneumoniae*, respectively. VRC-3406 also had an MIC of 8 μg/ml against the linezolid-resistant *S. aureus* mutant (linezolid MIC > 64 μg/ml). Versicor

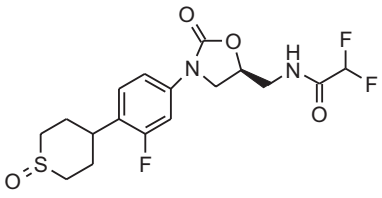
Figure 10: Chemical structures of some novel oxazolidinones.



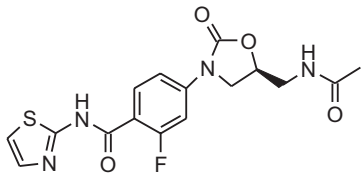
VRC-3816



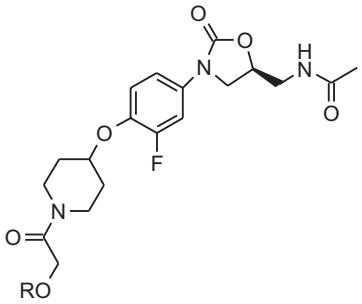
VRC-322



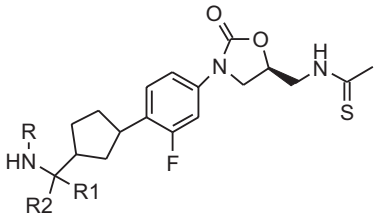
VRC-3839



VRC-3054



RWJ-302377, R=Ch$_2$Ph
RWJ-306490, R=H



PNU: 2-aminomethyl-1,3,4-thiadiazole phenyl oxazolidinones

has also identified novel 4-amido-3 fluorophenyloxazolidinones (MF Gordeev *et al.*, Versicor, Fremont, CA). VRC-3125 was the most active in this series with MIC values of 1 - 2, 1 and 2 μg/ml against *S. aureus*, VRE and *S. pneumoniae*, respectively. No *in vivo* efficacy was shown for VRC-3125, but VRC-3054 (**Figure 10**) and VRC-3055 were both found to be orally efficacious with ED_{50} values of 8.5 and 7.5 mg/kg/day in a murine model of septicaemia. These values are nearly identical to ED_{50} values resulting from treatment with linezolid. The activities of novel 4-acylamino and 4-sulfonamido-3-fluorophenyl oxazolidinones were also presented (MF Gordeev *et al.*, Versicor, Fremont, CA). VRC-3599 was the most active compound in this series with MIC values of 0.25 - 0.5, 8, 0.5, 0.13 - 0.5 μg/ml, against *S. aureus*, linezolid-resistant *S. aureus*, VRE and *S. pneumoniae*, respectively and MIC values of 2 - 8 and 4 μg/ml against the fastidious Gram-negative bacteria *H. influenzae* and *M. catarrhalis*, respectively. Versicor provided information on their synthesis and screening of novel tetrahydo-4(2H)-thiopyranyl sulphoxide and sulphone libraries. VRC-3816 and VRC-3839 (**Figure 10**) were active against the Gram-positive pathogens (MIC values of 0.5 - 4 μg/ml), active against the fastidious Gram-negative pathogens (MIC values of 4 - 8 μg/ml) and were also active in a murine model of septicaemia (ED_{50} values of VRC-3816 and VRC-3839 were 3.75 and 6.52 mg/kg/day, respectively).

Investigators at AstraZeneca found that the acetamidomethyl side chain could be replaced by limited 5- and 6-membered oxygen-linked heteroaromatic substituents and retain activity *in vitro* that was comparable with the amide analogues (MB Gravestock, AstraZeneca, Macclesfield, UK). These substitutions were made to oxazolidinones previously modified by having an unsaturated piperidine or

pyran at the C-4-position on the phenyl ring. Heterocycles containing sulphur were the most active. The thiadiazolyl series yielded MIC values of 0.13 - 0.25 μg/ml against *S. aureus* and 0.25 μg/ml against *E. faecalis*. *In vivo* efficacy was verified as a reduction in bacterial numbers in a murine model of a localised *S. aureus* infection. Investigators also found that the C-5 of the oxazolidinones (modified by having an unsaturated piperidine or pyran at the C-4-position of the phenyl ring) can be replaced by a wide variety of heterocyclic aminomethyl groups (RG Wilson, AstraZeneca, Macclesfield, Cheshire, England). The unsubstituted 5-membered rings provided the greatest amount of *in vitro* activity (MIC values of 0.13 - 0.5 μg/ml against the Gram-positive pathogens). This *in vitro* activity was significantly reduced by the increase in ring size to a 6-membered ring. Compounds with an isoxazole at C-5 provided the best combination of *in vitro* activity and *in vivo* efficacy. In continuing the search for the second-generation oxazolidinones, the investigators at AstraZeneca showed that O-linked and N-linked 5-membered heterocycles at the C-5-position can be combined with a wide range of N-linked heterocycles at the C-4 of the phenyl ring. The most active compounds in the 4-heteroaryl-1-piperazine series had MIC values of 0.06 - 0.5 μg/ml. In the 4-heteroaryl-1-piperazine series, the best compounds had MIC values of 0.125 - 0.5 μg/ml against the Gram-positive pathogens. The best compounds in the N-linked heteroaromatic series had MIC values ranging from 0.13 - 2 μg/ml against the Gram-positive pathogens and the most active compounds in the 3-aminopyrrolidine series had MIC values of 0.13 - 1 μg/ml. The *in vivo* efficacy of these compounds were verified in a murine model of a localised thigh infection. The thiadiazolyloxymethyl and the isoxazolaminomethyl groups were the most active *in vitro*. However, the most efficacious compounds *in vivo* were those with the amino linkage.

LM Thomasco *et al.* (Pharmacia Corporation, Kalamazoo, MI) provided *in vitro* and *in vivo* data on the 2-aminomethyl-1,3,4-thiadiazole phenyl oxazolidinone thioamides (**Figure 10**). Many compounds in this series had excellent activity against the Gram-positive pathogens (MIC values of 0.25 - 1 μg/ml) as well as activity against the fastidious Gram-negative pathogens *H. influenzae* and *M. catarrhalis* (MIC values of 0.5 - 41 μg/ml). However, none of these very lipophilic compounds were efficacious in a murine model of septicaemia (ED_{50} values > 20 mg/kg/day) when compared with linezolid (ED_{50} values of 2 - 9 mg/kg/day).

Investigations at Bayer on the imidazo-benzoxazinyl-oxazolidinones (S Bartel *et al.*, Bayer AG, Leverkusen, Germany and Bayer AG, Wuppertal, Germany) have shown that compounds in this series have very potent *in vitro* activity against respiratory pathogens including *S. pneumoniae, H. influenzae* and *M. pneumoniae*. MIC values for these active compounds were 0.125 - 4 μg/ml for *S. pneumoniae* and 0.5 - 8 μg/ml for *H. influenzae*. An N-acetyl derivative was selected for further study (R Endermann *et al.*, Bayer AG, Wuppertal, Germany and Bayer AG, Leverkusen, Germany). Although the pharmacokinetic parameters were less favourable with this compound compared with linezolid, the overall increase in *in vitro* activity resulted in an *in vivo* efficacy that was as good as or superior to linezolid. Both compounds significantly reduced lung titres of *S. pneumoniae* (3 $\log_{10}$ CFU/g tissue at 2 x 25 mg/kg/day, orally). The N-acetyl-derivative caused only a slight reduction in bacterial counts of *H. influenzae* in the murine respiratory model. In the murine model of septicaemia, the N-acetyl derivative was reportedly more efficacious than linezolid.

Efforts at the RW Johnson Pharmaceutical Research Institute (MA Weidner-Wells *et al.*, Raritan, NJ) focused on the modifications of the N-substituent of piperidinyloxy-substituted oxazolidinones (**Figure 10**). These compounds were observed to have moderate to weak activity against Gram-positive pathogens, with the best compounds having MIC values of 2 - 16 μg/ml.

9. Antifungals

9.1 Ravuconazole

Bristol-Myers Squibb presented a number of posters on their new broad spectrum antifungal agent, ravuconazole. SJ Olsen *et al.* (Bristol-Myers Squibb, Princeton, NJ and Medeval Ltd., Manchester, UK) described the clinical safety study of a single ascending oral dose in healthy patients. This clinical trial was a randomised, double-blind, placebo-dosed and single-dosed study; the dose was escalated in healthy male patients with the first dose of 50 mg oral capsule of ravuconazole or placebo. Patients were evaluated at days 4 and 7 for safety and tolerability. Eight untreated patients were then given escalation doses of 100, 200, 400, 600 and 800 mg. Clinical and

laboratory evaluations for the next four weeks were followed. A second dose was provided to the patients in the 100, 400 and 800 mg groups. The patients in the 200 mg groups received an oral solution of 10 ml of 20 mg/ml ravuconazole or placebo with clinical and laboratory evaluations for the following four weeks. The safety profile showed no serious adverse events with headache and abdominal pain the most frequently reported. Ravuconazole was 50% bioavailable at the 200 mg oral solution dose. A half-life ranging from 3.5 - 7.5 days was reported supporting once daily dosing regimen. DM Grasela *et al.* (Bristol-Myers Squibb and Medeval Ltd.) reported on the multiple ascending oral dose in healthy subjects. A sequential ascending dose study of ravuconazole with the following doses: 50, 100, 200 and 400 mg. Healthy male patients were treated once a day for 14 consecutive days, beginning with the lowest concentration group. Safety and tolerability were monitored for up to 28 days for each group. Each dose study began after the results of the lowest study was available. The most serious adverse event reported was headache. Ravuconazole was safe and well-tolerated up to the 400 mg dose. The half-life ranged from 4.3 - 10 days and continued to accumulate throughout the study. Ravuconazole evaluation of the cytochrome P450 isoenzyme, CYP3A, showed no induction.

9.2 Azasordarins

There were several presentations that provided information on the *in vitro* and *in vivo* activities of the Glaxo azasordarins. Azasordarins are derivatives of the sordarins that are characterised by a 6-methylmorpholi-2-yl group with N-4 substituents different from the sugar moiety. According to the data provided by E Herreros *et al.* (GlaxoWellcome S A, Madrid, Spain), GW 471588 and GW 531920, respectively, were found to have MIC_{90}s of 0.015 and 0.004 µg/ml against *Candida albicans*, 0.25 and 0.25 µg/ml against *C. glabrata*, 0.12 and 0.12 µg/ml against *C. tropicalis* and > 16 and 0.25 µg/ml against *C. parapsilosis*. *C. krusei* was resistant to both azasordarins. GW 531920 was the more active agent against *Aspergillus* and the dermatophytes, with MIC values of 16 µg/ml against *A. fumigatus*, 4 µg/ml against *A. flavus* and 0.12 µg/ml against *Trichophyton mentagrophytes*. It was also more active against the less common moulds, with MIC values of 2 µg/ml against *Fusarium oxysporum*. The development of resistance was also investigated by E Herreros *et al.* Resistance emergence was evaluated by sequential subculture in sub-inhibitory concentrations of amphotericin B, 5-flucytosine, fluconazole or GW 471558 and single step selection of resistance on agar containing 10 or 100 times MIC of one of the antifungal agents. No progressive increase in MIC values of GW 471558 occurred even after 40 serial passages. In single-step studies, the frequency of GW 471558 mutants on 10 times MIC was in the order of 2×10^{-8} or lower and similar to values obtained with amphotericin B. The frequency of mutation with 5-flucytosine was about three orders of magnitude greater than the frequency found for GW 471558. The efficacies of GW 471552, GW 471558 and GW 531920 were evaluated in two different rat models of *Pneumocystis carinii* infections (A Matinez *et al.*, Glaxo Wellcome S A, Madrid, Spain). In both models, doses of 0.25 - 5 mg/kg subcutaneously, twice a day for ten consecutive days, significantly reduced the number of *P. carinii* cysts and also strongly inhibited parasite development in infected rats compared with the untreated controls. Treatment of infected rats with trimethoprim/sulphamethoxazole proved ineffective. The *in vivo* efficacies of the azasordarins, GW 471552, GW 471558 and GW 531920 were also evaluated in rat models of oral and vaginal candidiasis at doses of 1, 5 and 10 mg/kg, subcutaneously, three times a day, for seven days or every 4 h for three days. In the rat model of oral candidiasis, all the azasordarins eradicated the infection at the 10 mg/kg dose. GW 471552 and GW 531920 at 5 mg/kg, markedly reduced the number of recoverable organisms compared with the untreated controls. In the rat model of vaginal candidiasis, GW 471558 and GW 521920 eradicated the organisms at 5 mg/kg. GW 471552 significantly reduced the number of recoverable organisms at 5 mg/kg and eradicated the organisms at 10 mg/kg. The therapeutic efficacy of GW 531920 was tested in a murine model of systemic candidiasis (P Aviles *et al.*, GlaxoWellcome S A, Madrid, Spain) at doses of 40, 20, 10 and 5 mg/kg, subcutaneously, every 8 h, for seven consecutive days. A pharmacokinetic analysis was performed as well to obtain information on dosing proportionality. Overall, there was a good correlation with the net effect *versus* daily dose and the percent survivors *versus* daily dose. Dose proportionality was also demonstrated. For the 40, 20, 10 and 5 mg/kg dose, the AUCs (µg.h/ml) were 63.4, 22.9, 12.2 and 5.6, respectively and the C_{max}s (µg/ml) were 29.9, 11.3, 5.2 and 2.13, respectively, while the percent survivors by day 18 were 90, 60, 20 and 0%, respectively. The pharmacokinetic parameters of GW 471558 and GW 531920 were evaluated following iv. administration in

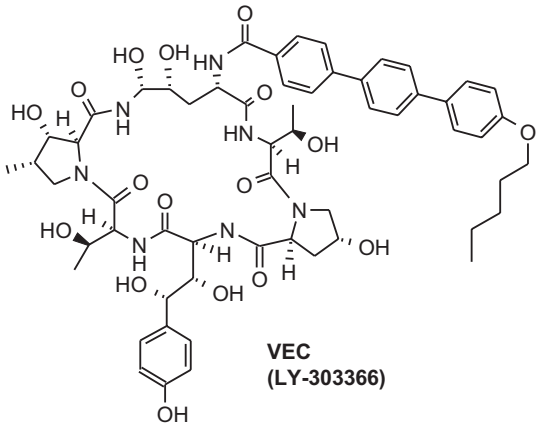
the mouse, rat and dog. Based on the resulting AUCs (μg.h/ml), $t_{½}$ and V_{ss} (l/kg), the investigators concluded that the azasordarins have PK properties that can be predicted across the different species. Preliminary toxicology of the azasordarins was also provided (E Herreros *et al.*) for GW 471552 and GW 471558. Cytotoxicity was tested against five different cell lines and a primary hepatocyte cell line. In the transformed cell lines from target organs including brain, cervix, muscle, kidney, lung and liver, cytotoxicity was evaluated by crystal violet exclusion and protein synthesis inhibition at 48 and 72 h. For the primary hepatocyte line, toxicity was evaluated by intracellular lactic dehydrogenase. For GW 471552, IC_{50} values ranged from 62 to > 128 μg/ml and for GW 471558 the IC_{50} values (μg/ml) were all > 128. In considering the activity of the azasordarins against *C. albicans*, this represents a therapeutic index of ~10,000. The LD_{50}s were also evaluated against male and female mice following a bolus (10 - 15 sec) iv. administration. The LD_{50}s (mg/kg) for GW 471552 were 196 (male) and 276 (female) and the LD_{50}s for GW 471558 were 222 (male) and 268 (female).

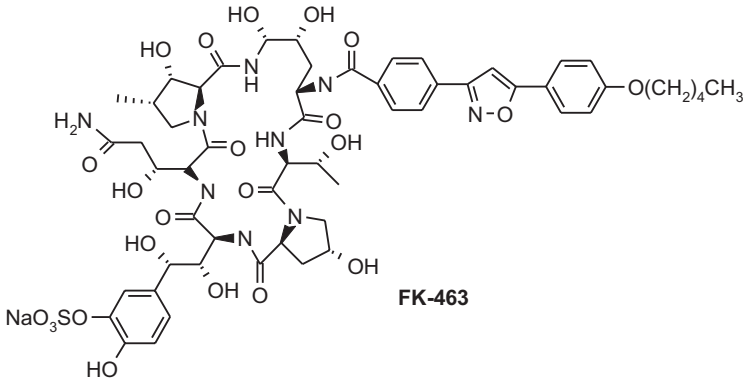
9.3 Echinocandins

CM Douglas *et al.* (Merck, West Point, PA and Thomas Jefferson University, Philadelphia, PA) presented on the activity of caspofungin against *Aspergillus fumigatus in vitro* and *in vivo* in immunosuppressed mice. The murine cyclophosphamide-induced immunosuppression produced a chronic immunosuppression when mice were treated with cyclophosphamide over the course of the experiment (28 days) or a transient immunosuppression when treatment was over 14 days. Mice in the chronic immunosuppression murine model were treated once daily with either caspofungin or amphotericin B for seven days. The PD_{50}s were 0.173 and 0.235 mg/kg for caspofungin and amphotericin B, respectively. Mice in the transient immunosuppression model were treated once daily for 14 days, producing PD_{50}s of 0.245 and 0.264 mg/kg for caspofungin and amphotericin B, respectively. The survival of mice in both immunosuppressed murine models was 100%. The effect of caspofungin at a dose of 0.3 μg/ml for 6 h at 37°C against *A. fumigatus* in liquid culture showed killing of the apical cells. The killing capacity of caspofungin of the tips of *A. fumigatus* hyphae grown in liquid cultures was 75%.

Versicor, Inc. (Fremont, CA) presented data from their Phase I and Phase II trials of V-echinocandin (VEC, formerly LY-303366) (**Figure 11**), a semi-synthetic antifungal agent under development for the iv. treatment of serious systemic fungal infections due to *Candida* and *Aspergillus*. The echinocandins are fungicidal for *Candida* and act by inhibiting β-1,3 glucan synthase. The Phase I dose optimisation studies (GL Brown *et al.*, Versicor, Inc. and Charterhouse Clinical Research Unit, Royal Masonic Hospital, London, England) showed that VEC could be dosed at a higher concentration (loading/maintenance) than tested in the earlier Phase I. Doses tested in this study were 100/70 mg (cohort A) and 140/100 mg (cohort B). VEC was well-tolerated in 5/6 patients given the 100/70 dose and in 3/6 patients given the 140/100 dose. Patients in both cohort A and B who did not tolerate the drug well exhibited transient symptoms consistent with a histamine release type of reaction. In cohort B, 2/3 of these patients also exhibited a grade II adverse event. Based on the PK and safety data provided, the investigators believe that the 100/70 would be the maximum tolerated dose and that Phase II/III trials can be conducted safely at doses higher than the previously reported 70/35 mg regimen. The Phase II, randomised, open-label study, consisted of 36 patients with oesophageal candidiasis given VEC in either of two iv. doses, 50/25 mg (loading/maintenance) or 70/35 mg, for a maximum of 14 - 21 days (GL Brown *et al.*, Versicor, Fremont, CA and Eli Lilly and Company, Indianapolis, IN). At the termination of the study, 29/36 patients were considered evaluable, 16 in the 50/25 mg group and 13 in the 70/35 mg group. Endoscopy scores based on achieving a grade 0 or 1 improvement at the end of treatment gave an efficacy of 81% for the 50/25 mg group and 85% for the 70/35 mg group. In the 50/25 mg group, 68.8% (11/16) had resolution of both dysphagia and retrosternal chest pain and in the 70/35 mg group, 81.8% (9/11) had resolution of both dysphagia and retrosternal chest pain. Of all 36 patients enrolled, there were a total of 28 adverse events in 16 patients (eight patients/cohort) that were reported as possibly related to the study drug, including injection site reaction (three), headache (three), vasodilation (two), hypotension (two) and nausea (two). There were 11/36 patients (31%) that experienced a serious adverse event; three were reported as possibly related to study drug and included aspartate amino transferase (serum glutamic oxaloacetic transaminase-SGOT) increase, alanine amino transferase (serum glutamic pyruvic transminase-SGPT) increase and leukopoenia.

Figure 11: Chemical structures of the echinocandins, VEC and FK-463.





Several presentations also highlighted the development of FK-463, the echinocandin under development by Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan (**Figure 11**). The *in vitro* activity of FK-463 was evaluated against a spectrum of *Candida* species (L Ostrosky-Zeichner *et al.*, Univ. of Texas, Houston, Medical School, Houston, TX). The values were essentially identical after 24 or 48 h of incubation, whether evaluated visually or spectrophoto metrically. The median MIC (range) was 0.015 µg/ml (0.015 - 2 µg/ml) for *C. albicans* (272), 0.015 µg/ml (0.015 - 2 µg/ml) for *C. glabrata* (94), 0.25 µg/ml (0.015 - 0.5 µg/ml) for *C. krusei* (32), 0.062 µg/ml (0.031 - 1 µg/ml) for *C. lusitaniae* (11), 1 µg/ml (0.015 - 4 µg/ml) for *C. parapsilosis* (104) and 0.031 µg/ml (0.015 - 1 µg/ml) for *C. tropicalis* (92). V Petraitis *et al.* (NCI, NIH, Bethesda, MD) presented a study that involved the comparative antifungal activity of FK-463 against disseminated candidiasis and invasive pulmonary aspergillosis in persistently neutropoenic rabbits. Rabbits with candidiasis were treated intravenously with FK-463 at 0.25, 0.5 and 1 mg/kg or amphotericin B at 1 mg/kg 24 h following infection. There was a dosage-dependent clearance of organisms. In rabbits treated with 0.25 mg/kg FK-463, there was clearance of *C. albicans* in all tissues except the vena cava and lungs. *In vitro* time-kill studies also showed a concentration-dependent killing of *C. albicans*. Rabbits infected with *A. fumigatus* were treated intravenously with FK-463 at 0.5, 1, or 2 mg/kg, amphotericin B or liposomal amphotericin B. Although there was an increase in survival and a decrease in infarcts, there was not a dosage-dependent reduction in the CFU/g tissue of *A. fumigatus* in this model. As expected, the rabbits treated with amphotericin B had significant reductions of *C. albicans* and *A. fumigatus* in both of these models. In a murine model of aspergillosis (M Nakajima *et al.*, Kawasaki Medical School, Kurashiki, Japan and Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan), mice were treated with FK-463 (0.5, 1, or 2 mg/kg), amphotericin B (0.25 or 0.5 mg/kg) or a

combination of FK-463 and amphotericin B at 1 and 0.25 mg/kg or 2 and 0.5 mg/kg. The investigators concluded that pathological findings in the mice treated with the combination of amphotericin B and FK-463 were more favourable and correlated with improved survival rates compared with the two groups treated with FK-463 or amphotericin B alone. Other investigators (S Kohno *et al.*, Nagasaki Univ. School of Medicine, Nagasaki, Japan and Saitama Medical School, Saitama, Japan) who also used a similar murine model of aspergillosis, found the combination of FK-463 plus amphotericin B effective (more effective than either agent alone) and the combination significantly reduced the fungal burden in the lungs of mice.

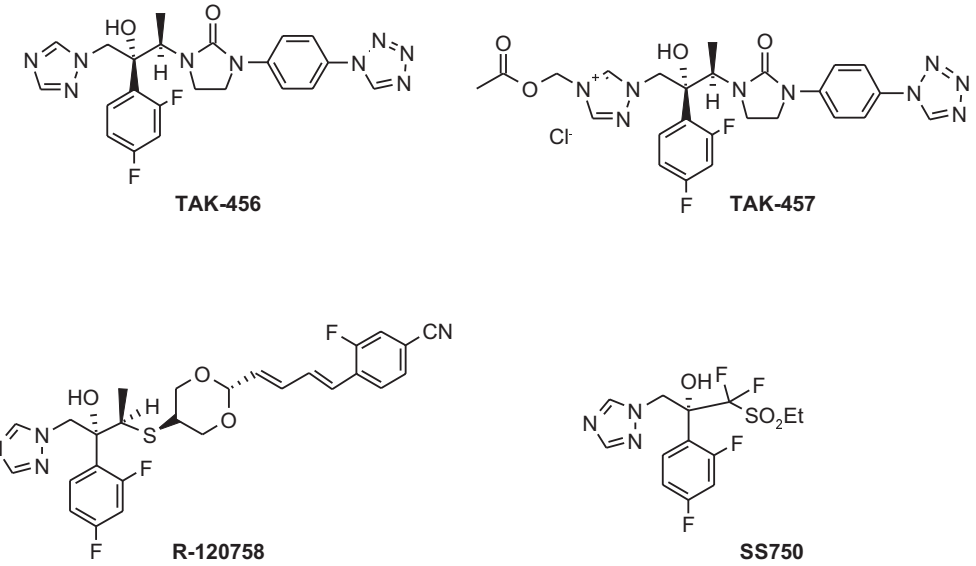
The pharmacodynamics of FK-463 were evaluated in a neutropoenic mouse thigh infection induced by injection of *C. albicans*. Mice were treated with 0.5, 1 and 2 mg/kg FK-463 and viable counts in the muscle were evaluated 24 h after therapy. Mice were treated 1 h following infection. The reduction in viable CFU/g showed that there was a dose-dependent response to treatment with FK-463 and at 1 mg/kg there was a significant reduction in CFU/g compared with untreated controls. The concentration of FK-463 in muscle at 1 and 7 h following therapy with FK-463 at 1 mg/kg was 0.5 and 0.3 μg/ml, respectively. These values are equivalent to 0.5 and 0.25 times the MIC in 90% mouse serum or 4% human serum, respectively and were very much greater than the MIC values determined in RPMI/MOPS. The plasma pharmacokinetics and tissue distribution of FK-463 were evaluated in rabbits (AH Groll *et al.*, NCI, Bethesda, MD, NIH, Bethesda, MD, MDS, Harris, Lincoln, NE and Fujisawa Healthcare, Deerfield, IL). FK-463 was dosed at 0.5, 1 and 2 mg/kg iv. into normal rabbits once a day for eight consecutive days. The C_{max} values were 10.3, 17.2 and 19.7 μg/ml and the AUC values were 5.7, 13.4 and 22.9 μg.h/ml, respectively. For all three doses, the VD was ~ 0.3 l/kg, the Cl was 0.8 - 0.9 l/h/kg and the $t_{½}$ values were ~ 3 h. The plasma pharmacokinetics were best described by a two-compartment pharmacokinetic model that showed linear disposition. FK-463 achieved potentially therapeutic levels that exceeded the MIC of susceptible fungi. FK-463 also penetrated into tissues that are common sites of fungal infection. Tissue concentrations near peak plasma concentrations were observed in lung, liver, spleen and kidney.

9.4 Novel azoles

S Takeda *et al.* (SSP Co. Ltd., Chiba, Japan) presented the structure-activity relationship of SS750, a new triazole containing a *gem*-difluormethylsulphonyl moiety (**Figure 12**). Compounds were evaluated for *in vitro* activity against various fungal pathogens (yeast and moulds) and were tested for *in vivo* activity against a murine model of systemic candidiasis. The most active agent, SS750, contained an ethanol substituent at R′. SS750 had MIC values of 0.063 μg/ml for *C. albicans*, 0.5 μg/ml for *C. krusei*, 2 μg/ml for *A. fumigatus* and 2 μg/ml for *A. flavus*. This compound was also efficacious *in vivo* with 5 of 5 mice surviving infection following treatment with oral SS750 at 1.25 mg/kg/day. Studies by M Matsumoto *et al.* (SSP Co. Ltd., Chiba, Japan), were established to compare the *in vitro* and *in vivo* activities of SS750 with the activities of fluconazole and itraconazole. *In vitro*, SS750 was more active than fluconazole, but less active than itraconazole against a fluconazole-susceptible and -resistant strain of *C. albicans*.

When evaluated for *in vivo* efficacy (on day 7) against the fluconazole-susceptible strain, SS750 given orally was four- to five-fold more active than fluconazole and 100- to 200-fold more active than itraconazole in a murine model of systemic candidiasis (involving either normal or immunosuppressed mice) as well as in a murine model of pulmonary candidiasis. When given intravenously, SS750 was four- to five-fold more efficacious than fluconazole given intravenously. Given orally, SS750 on day 7 was four-fold more active than fluconazole and > 16-fold more active than itraconazole against the fluconazole-resistant strain. When given intravenously, SS750 was > ten-fold more active than fluconazole given intravenously. The therapeutic efficacy of SS750 was also evaluated in murine models of systemic aspergillosis and cryptococcosis (K Yokoyama *et al.*, Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba, Japan and SSP Co. Ltd., Chiba, Japan). The *in vitro* activity against the strains used in these models showed SS750 to be more active than fluconazole, but less active than itraconazole. However, SS750 had excellent efficacy *in vivo*, with equivalence shown for iv. and oral administration, against systemic aspergillosis and cryptococcosis in immunosuppressed mice. In both models, SS750 was significantly more efficacious than either fluconazole or itraconazole. T Kogure *et al.* (SSP Co. Ltd., Chiba, Japan) investigated the metabolic fate of SS750 in rats. Rats were dosed with radiolabelled SS750, orally and intravenously, at

Figure 12: Chemical structures of some novel azoles.



4 mg/kg. A C_{max} of ~ 2 μg/ml in plasma was achieved in ~ 1 h. Plasma concentrations were reduced with a $t_{½}$ of 2 and 1.4 h for the iv. and oral administration, respectively. Bioavailability achieved ~ 75% and protein binding was 50 - 63%. At 1 and 4 h post-administration, SS750 was found mainly unchanged in the liver and plasma. The high bioavailability and high absorption support the observed *in vivo* efficacy. The effects of SS750 on cytochrome P450 were studied by K Ishida *et al.* (SSP Co. Ltd., Chiba, Japan). The IC_{50} values of SS750 for inhibition of [^{14}C] mevalonic acid into ergosterol of *C. albicans* and *C. krusei* were equivalent to the IC_{50} values of itraconazole at 1.5- and 3.7-fold, respectively and less than fluconazole. SS750 was a more potent inhibitor of the fungal cytochrome P450 system than fluconazole, however, the affinity for the human cytochrome P450 was equivalent for SS750 and fluconazole. These data demonstrate a higher selectivity between the fungal and human cytochrome P450 systems for SS750 compared with fluconazole.

The *in vitro* and *in vivo* antifungal activities for TAK-456 and the water soluble prodrug TAK-457 (**Figure 12**) were reported (T Kitazaki *et al.*, Takeda Chemical Industries Ltd, Osaka Japan). TAK-456 is a new azole that contains an imidazolidinone nucleus. TAK-456 has activity against *C. albicans* (MIC values, 0.016 - 0.03 μg/ml), azole-resistant *C. albicans* (MIC, 2 μg/ml), *C. glabrata* (MIC, 4 μg/ml), *C. krusei* (MIC, 1 μg/ml), *Cryptococcus neoformans* (MIC, 0.5 μg/ml), *A. fumigatus* (MIC, 0.5 μg/ml) and *A. flavus* (MIC, 1 μg/ml). Following oral administration, TAK-456 was found have an ED_{50} of 1.6 - 2.3 μM/kg in a murine model of systemic candidiasis. However, TAK-456 had a water solubility of 5 μg/ml. Quaternary salts of TAK-456 were synthesised to identify a water-soluble prodrug for injectable formulation. TAK-457, which contained an acetoxymethyl triazolium moiety, had water solubility (4 - 10 mg/ml) and stability (*in vitro* $t_{½}$ of 5 - 6.4 h in mouse, rat and human) sufficient for an iv. formulation. In mouse, rat and human plasma *in vitro*, TAK-457 was readily converted to TAK-456 and it was also readily converted to TAK-456 in rats. Further studies on the *in vitro* activity and *in vivo* efficacy were provided by Y Iizawa *et al.* (Takeda Chemical Industries Ltd, Osaka, Japan). Both TAK-456 and TAK-457 were found to be more active than fluconazole, itraconazole and voriconazole in murine models of systemic candidiasis in normal and neutropoenic mice, whether the infecting strain was fluconazole-susceptible or fluconazole-resistant. TAK-457 was also more active than amphotericin B in a murine model of an *A. fumigatus* pulmonary infection when survival rates, lung weights, reduction of fungal burden in lungs and plasma β-glucan levels were all compared. TAK-456 and TAK-457 may prove useful in the treatment of fungal infections including those caused by *Aspergillus*.

The *in vitro* activity of another newer triazole, R-120758 (**Figure 12**), against fungal clinical isolates (50 strains of each species) was also presented (A Sanchez *et al.*, Harbor-UCLA Research and Education Institute, Torrance, CA and Sankyo Co. Ltd, Tokyo, Japan). R-120758 had MIC_{50}/MIC_{90}s of ≤ 0.004/0.016

μg/ml against *C. albicans*, 0.5/≥ 4 μg/ml against fluconazole-resistant *C. albicans*, 0.5/2 μg/ml against *C. glabrata*, 0.25/0.25 μg/ml against *C. krusei*, 0.016/0.016 μg/ml against *C. parapsilosis*, 0.016/0.5 μg/ml against *C. tropicalis* and ≤ 0.004/≤ 0.004 μg/ml against *C. neoformans*. Continued development plans were indicated for this triazole antifungal.

10. Efflux pump inhibitors

10.1 Bacterial

Microcide had an entire poster session devoted to its work on efflux pump inhibitors (TE Renau *et al.*, R Leger *et al.*, MS Warren *et al.*, D Griffith *et al.*, D Cho *et al.*). The antibacterial efflux inhibitors were derived from a dipeptide structure, which had been modified with N-methyl groups to stabilise the derivative to serum peptidases. The modified compounds were found to be slightly less active in potentiation of levofloxacin against efflux pump overexpressing pseudomonads *in vitro*, but stable to human serum for an extensive period of time. The resulting compound, D-ornithine-D-homo-phenylalanine-3NHQ, was used as a basis for further modification by substitutions, which led to peptidomimetic versions of the inhibitor. Classes made and evaluated included ether and thioether analogues, which were very active in potentiation. Benzoxazole derivatives also had good potentiation, but benzimidazoles and benzthiazoles were less active.

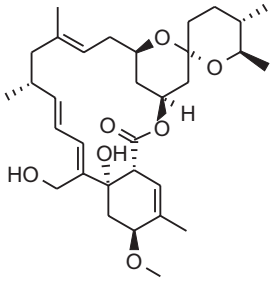
Another presentation focused on the inhibitor MC 207110 (**Figure 13**), identified from high throughput screening of a library of efflux inhibitor candidates based on the prototypes. When tested against the Mex pumps of *P. aeruginosa*, the compound was found to be an inhibitor of the RND family of efflux pumps and antibiotics that are effluxed by the pump do not compete with MC 207110. This compound also permeabilises the outer membrane of *P. aeruginosa*. Testing of the dipeptide efflux inhibitor MC 02595 in mouse infection models with *P. aeruginosa* (MexCD, OprJ overexpression strain) demonstrated an effect on the ED_{50} values in the sepsis model, consistent with the lowered *in vitro* MIC values. A similar result was obtained in the reduction of bacteria isolated from infected thigh muscle in the mouse by the combination of efflux inhibitor and levofloxacin *versus* levofloxacin alone. Another *in vitro* study with a variety of Gram-negative organisms (*E. coli*, *Salmonella typhimurium*, *H. influenzae*, *Acinetobacter baumanii* and *P. aeruginosa*) demonstrated an effect on the MIC_{90} values against azithromycin, clarithromycin and erythromycin with the efflux inhibitor MC 04124.

10.2 Fungal EPIs

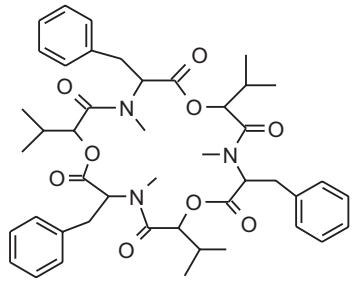
M Warren *et al.* (Microcide Pharm. Co. and Inst. Of Microbiology, Lausanne, Switzerland) presented a poster on inhibitors of fungal efflux pumps. There were two types of inhibitors, one broad spectrum targeting ABC transporters, CDR1 and CDR2 and the second narrow spectrum targeting the CDR1 pump. Two broad spectrum inhibitors, milbemycin and beauvericin (**Figure 13**), given in combination with either fluconazole or terbinafine, inhibited CDR1 in both *C. albicans* and *C. glabrata*, decreasing the MIC 16- to 128-fold. Beauvericin had no effect on potentiating fluconazole against *C. albicans* with BEN upregulated. BEN is an MDR pump of the major facilitator family and not effected by the inhibitors. In addition, beauvericin had no effect against defined strains of both *C. albicans* and *C. glabrata* with deleted copies of CDR1 and CDR2. This showed specificity for these pumps by its lack of effect on the strains with the deleted pumps. The narrow spectrum efflux pump inhibitors, MC 05,204 and MC 380,286, treated at a concentration of 1 μg/ml with fluconazole, ketoconazole, itraconazole or posaconazole resulted in a decrease of MIC values ranging from 133- to 256-fold. Microcide's inhibitors are effective against ABC transporters, either broad spectrum, CDR1 and CDR2 or narrow spectrum, CDR1 only and no effect has been shown for the major facilitator family.

Another poster presented by K Sorensen *et al.* (Microcide Pharm. Co.) described the *in vivo* potentiation of fluconazole by MC 510,011 (**Figure 13**). A murine neutropoenic mouse model and two isogenic pairs of *C. albicans*: YEM 14 fluconazole-susceptible and YEM 15 overexpressing CDR1 and CDR2. The compounds evaluated were MC 510,011 a broad spectrum efflux pump inhibitor (CDR1 and CDR2), treated at a dose of 2 and 200 mg/kg orally and fluconazole treated at a dose of 3.1 and 100 mg/kg, intraperitoneal. Against the strain YEM 14, MC 510,011 decreased the kidney fungal burden by 2.0 - 2.5 $\log_{10}$ CFU/g. In contrast to strain YEM 15, neither MC 510,011 or fluconazole (100 mg/kg) had an effect on decreasing kidney fungal burden. Combination treatment of fluconazole and MC 510,011 decreased kidney fungal burden by 1 - 2 $\log_{10}$ CFU/g. Assessment of kidney fungal burden showed that the

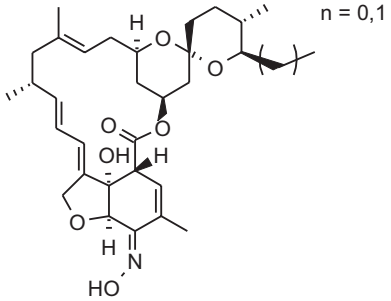
Figure 13: Chemical structures of some efflux pump inhibitors.



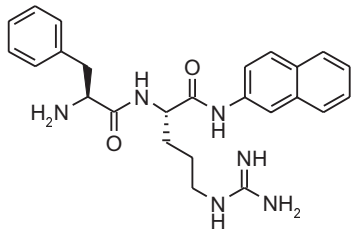
Milbemycin (MC-510,021)



Beauvericin (MC-510,065)



MC-510,011



MC-207110

fluconazole and MC 510,011 combination had a 26% decrease for YEM 14 and 36% decrease for YEM 15 over fluconazole treatment alone. The pharmacokinetic profile of MC 510,011 showed no effect in infected neutropoenic mice. The effect on fluconazole MIC values by treatment with MC 510,011 at a dose ranging 0 to 4 μg/ml was a 0- to 133-fold decrease in MIC for YEM 14 and a 0- to 1032-fold decrease in MIC for YEM 15. The poster suggested that combination therapy of an azole and an efflux pump inhibitor maybe a useful approach in overcoming azole resistance.

11. Exploratory research

11.1 Resistance to ketolides: role of ribosomal modification and macrolide efflux

Ketolides are 14-membered ring macrolides, which have a 3-keto group instead of an L-cladinose moiety on the erythronolide A ring (R Leclercq, Hopital Cote de Nacre, Caen, France). They are distinguished from azalides, which have an N-modification of the rings. Ketolides are weak inducers of the *erm* methylase due to the lack of the cladinose moiety, including telithromycin, ABT-773 and TE-802. Global structure of the drug is important for induction of resistance, which can be conveniently measured by a green fluorescent reporter (GFP) on the methylase gene. As far as mechanism of action, the prototype of this class, erythromycin, interacts with the peptidyl transferase and methylation of the ribosome leads to decreased affinity of erythromycin. In the case of the ketolide ABT-773, there is improved binding to methylated ribosomes, albeit not enough to defeat resistance in MLS constitutive strains. In streptococci, however, MLS resistance is primarily inducible, therefore ketolides are active.

Other types of macrolide resistance include the efflux pump, *mefA*. This pump uses proton motive force for energy and telithromycin is a weak inducer and substrate relative to erythromycin. Another efflux pump is the *msrA*, which is an ABC transporter, is inducible and can result in a 6 - 12 times MIC increase. In pneumococci mutations in the stem-loop region that controls the methylase expression can destabilise the region, leading to low level expression of methylase and low level resistance. Additional

pneumococcal mutations are in the L22 protein, which lead to modest increases in the MIC of telithromycin. Mutations in L4 can give similar types of increases. These latter two, combined with the stem-loop mutation can result in resistant pneumococci.

11.2 Fluoroquinolone resistance in pneumococci

An overview of several aspects of fluoroquinolone (FQ) interactions with *S. pneumoniae* was described by LM Fisher (St. George's Hospital, London, England). These include efflux, with the *pmrA* encoded pump and target changes; namely DNA gyrase and topoisomerase IV gene mutations that lead to resistance. The two enzymes that are FQ targets, DNA gyrase and topoisomerase IV, both perform their functions *via* double-stranded cleavages in the DNA. Changes in the protein sequences that lead to increased antibiotic resistance are found in regions, originally identified in *E. coli* as QRDRs, which are hot spots for resistance development. The QRDR regions are highly similar in amino acid composition in both gyrase and topoisomerase, with resistance changes occurring largely in conserved amino acids. The N-terminal portion of the gyrase of *E. coli* has been crystallised and there is a helix-loop-helix domain that recognises the DNA, with tyrosines at the active site forming covalent bonds with the DNA during strand cleavage. The enzyme has a central hole where the active site resides, with 'gates' on either side for DNA entry and exit. The resistance mutational hot spots lie along an α-helix at the interface that interacts with DNA.

There are three types of common inhibition mechanisms for FQs, as distinguished by their *in vivo* target affinities as defined by mutant selection and genetic studies. The first, ciprofloxacin type, has topoisomerase IV as its primary target. The second type, sparfloxacin type, has DNA gyrase as a primary target and clinafloxacin defines the third type with approximately equal sensitivity of both DNA gyrase and topoisomerase IV to the drug. When measured with an *in vitro* biochemical assay, generally all FQs are more active against pneumococcal topoisomerase IV. With new compounds, one can quickly characterise them by performing MIC values with a panel of strains having mutations in DNA gyrase and topoisomerase IV. Many of the newer FQs (e.g., gatifloxacin, grepafloxacin, moxifloxacin, sparfloxacin) target GyrA. In contrast, topoisomerase IV appears to be the primary target of ciprofloxacin, norfloxacin, levofloxacin and trovafloxacin. Both single and double mutants of FQ targets have been detected in clinical isolates of pneumococci.

He then described work in which the four proteins (GyrA, GyrB, ParC and ParE) of pneumococci were his-tagged and overexpressed for *in vitro* assays. There are three types of assays that can be performed with these enzymes: supercoiling, decatenation and cleavable complex. Fisher indicated that the cleavable complex assay was the best indication of activity. He also cautioned against using IC_{50} values, as quinolones trap the enzyme on the DNA and induce a double-stranded DNA break, therefore it is not similar to a standard enzyme substrate reaction. In studies, both ciprofloxacin and sparfloxacin were found to trap cleavable complexes of gyrase and topoisomerase IV. The results from genetic (mutant selection) and biochemical assays do not always agree. For example, in pneumococcal topoisomerase IV is more susceptible in cleavable complex assay to all FQs compared with gyrase. Some quinolones have enhanced affinity for their targets, for example, the CC_{50} for ciprofloxacin against DNA gyrase is 80 and 1 μM against topoisomerase IV, whereas for clinafloxacin the values are 2.5 and 0.1 μM. To conclude with, Fisher presented a model for FQ action, in which DNA gyrase and topoisomerase IV represent two arms that can both lead to cleavable complexes. Subsequent to the establishment of a cleavable complex, there are double-stranded DNA breaks mediated by helicases and DNA polymerases involved in DNA replication, resulting in cell killing. There is competition between the two enzyme arms in the cell and there are downstream events that contribute to killing. When one performs an *in vitro* assay, one is not measuring these events, which explains the lack of correlation observed between *in vitro* enzyme assays and *in vivo* FQ killing.

11.3 Antibiotics and ribosomes: interaction and resistance

J Hansen (Yale Univ., New Haven, CT) described the 3-D structure of the ribosome. The recent solution of the 50S subunit at the 2.4 Å level by the Steitz laboratory has led to several major insights in peptide bond formation. The major achievement was the unequivocal demonstration that the peptidyl transferase is a ribozyme activity of the central loop in domain V of 23S rRNA. Implicated in this mechanism was the base of A 2451 in *E. coli*, which has an unusual pKa, due to hydrogen bond interactions with G 2447

and the buried phosphate of A2450. This allows the A 2451 to exist in a rare imino form and act in a charge relay that increases the negative charge density, allowing it to function as a base to facilitate a nucleophilic attack by the α-amino acid of the A-site substrate. Another feature of the ribosome is the existence of a 15 Å wide and 100 Å long tunnel, which leads from the peptidyl transferase site to an exit point for the nascent polypeptide. The ribosomal proteins were found to be largely globular, with long extensions that threaded into the ribosome and interacted with the ribosomal RNA to neutralise charges, fill voids and presumably stabilise the structure. It was found that none of the protein sidechains that penetrate and interact with domain V are closer than 18 Å to the peptidyl transferase site.

11.4 Novel 3-dithiocarbamoylcephalosporins

A number of novel 3-dithiocarbamoylcephalosporins (PGE-9951357, PGE-856854, PGE-9882816, PGE-9739390 and PGE-6737410) were presented by researchers from Procter & Gamble Pharmaceuticals (P&G, Mason, OH). Their compounds were among a series of compounds synthesised to identify new β-lactams with broad spectrum activity against multi-resistant Gram-positive bacteria including MRSA and PRSP, through the inhibition of multiple PBPs including PBP2a and PBP2x^R. The drug resistance in MRSA and PRSP was attributed in part to the presence of low affinity penicillin binding proteins (PBP2a and PBP2x^R, respectively). Synthesis and SAR study were provided. PGE-9951357 and PGE-856854, both possessing the 3-(isoindolinyl) dithiocarbamoyl moiety, had activities against MRSA (MIC values, 8, 64 μg/ml) and PRSP (MIC values, 1, 0.5 μg/ml) (RE White *et al.*, P&G). Introduction of a 5-chloro substituent to the aminothiazolyl(oximino)acetyl led to compounds with enhanced anti-MRSA/PRSP and PBP2a/PBP2x^R activity (e.g., PGE-6737410 and PGE-9882816) (Z Chen *et al.*, P&G). The potency of these compounds was reduced in the presence of fetal bovine serum (FBS). Efforts toward changing the physical properties by attaching an amino side chain to the isoindolinyl moiety resulted in compounds (e.g., PGE-9739390) with maintained *in vitro* potency, but reduced solubility. MIC values (μg/ml) for PGE-9882816, PGE-9739390 showed the following properties: MRSA, 2, 2; PRSP, ≤ 0.125, 0.5; *E. coli*, 2, 0.5; vancomycin-resistant *E. faecium*, 32, 8. IC_{50} values (μM): PBP2a, 0.9, 1.5; and PBP2x^R, 0.3, 0.15, respectively. The *in vivo* efficacy of PGE-9951357, PGE-9739390 and PGE-6737410 was evaluated in a murine sepsis model of infection. The 3-dithiocarbamoyl(carba)cephalosporins protected mice against infection by *S. pneumoniae* and *S. aureus*, but did not protect mice against infection by *E. coli* or methicillin-resistant, ciprofloxacin-resistant *S. aureus*.

11.5 R-115685

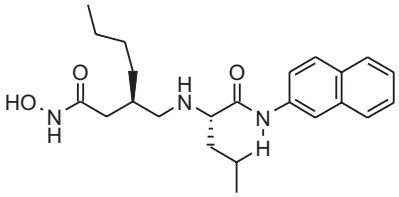
R-115685 is a new parenteral carbapenem antibiotic being developed by Sankyo Co. Ltd. (Tokyo, Japan). This 1-β-methylcarbapenem possesses excellent Gram-positive and Gram-negative antibacterial activity *in vitro*, including activity against MRSA, PRSP and imipenem-resistant *P. aeruginosa* (S Ohya *et al.*, Sankyo Co. Ltd., Tokyo, Japan). R-115685 is stable to dihydropeptidase I and appears to be less nephrotoxic than meropenem (I Kawamoto *et al.*, Sankyo Co. Ltd.). R-115685's balanced spectrum has the meropenem-like Gram-positive activity (S Ohya *et al.*, Sankyo Co. Ltd.). R-115685 has a 16-fold lower MIC than meropenem or imipenem against resistant mutants of *P. aeruginosa*, was stable against all but the metallo-β-lactamases and is bactericidal (N Masuda *et al.*, Sankyo Co. Ltd.). *In vivo* efficacy confirmed the activity of R-115685, with excellent protection against systemic infections caused by *Staphylococcus epidermidis*, *S. aureus*, *S. pneumoniae* and *Enterobacteriaceae*, with ED_{50} values of ≤ 8 mg/kg (N Masuda *et al.*, Sankyo Co. Ltd.). PK results in animals, extrapolated to humans, predicts less frequent dosing that with other carbapenems (S Ohya *et al.*, Sankyo Co. Ltd.).

12. Novel bacterial targets

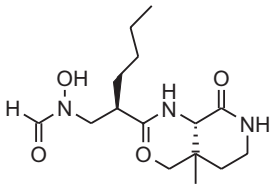
12.1 Peptide deformylase

Peptide deformylase is an essential bacterial enzyme, which is highly conserved. Versicor, in collaboration with Novartis, has synthesised combinatorial libraries of peptide deformylase (PDF) inhibitors. CJ Hackbarth *et al.* (Versicor, Fremont, CA and Novartis Pharma AG Summit, NJ) presented a poster on compounds that were screened against the PDF zinc metalloenzyme and a panel of Gram-positive and Gram-negative organisms. Several compounds showed improved antibacterial activity, while maintaining excellent enzymatic activity. VRC-3324 (**Figure 14**) was identified as a promising lead, with improved whole cell activity against *S. pneumoniae*,

Figure 14: Compounds for novel bacterial targets.



VRC-3324



BB-3497

S. pyogenes, *H. influenzae* and *M. catarrhalis* with MIC values ranging from 0.13 to 4 μg/ml.

Other lead compounds were VRC-3375, VRC-3488, VRC-3896 and VRC-3997 with excellent enzymatic activity, ranging from 1 - 8 nM and whole cell activity against some Gram-positive organisms. Bactericidal studies with these compounds determined them to be bacteriostatic according to the results of *in vitro* time-kill studies. Several other posters provided a profile of VRC-3375 (IC_{50} = 2 nM against Ni-PDF *E. coli*) a potent deformylase inhibitor. P Margolis *et al.* (Versicor) presented mechanisms of resistance studies against *H. influenzae* and *S. pneumoniae*. The rate of resistance development to VRC-3375 for both organisms was 10^{-8}, which is 100-fold less than *S. aureus*. A single functional deformylase gene is present in *H. influenzae*, unlike *S. pneumoniae* and *S. aureus*, which has two copies, with only one copy functional (defB). Resistance to *H. influenzae* showed frameshift mutations in formyltransferase (FMT), which results in the bypass of the formylation/deformylation cycle. Identifying the resistance mechanism for *H. influenzae* to be the same as *S. aureus*. Resistance to *S. pneumoniae* was identified as mis-sense mutations in defB. The resistant mutants to *H. influenzae* and *S. pneumoniae* showed antibiotic resistance to actinonin and VRC-3375, but a pattern of antibiotic susceptibility to other classes of antibiotics. This shows a resistance pattern specific to peptide deformylase compounds. D Chen *et al.* (Versicor) presented a poster on *in vivo* activity of VRC-3375. Pharmacokinetics of VRC-3375 was determined in mice at a dose of 100 mg/kg by iv., sc. and po. This compound has 64% oral bioavailability, it is rapidly absorbed from the gastrointestinal tract and distributed to various tissues and rapidly cleared from the serum. A mouse septicaemia model with *S. aureus* (Smith strain) was used to determine efficacy. VRC-3375 has PD_{50} values of 32 mg/kg iv., 17 mg/kg sc. and 21 mg/kg po.

British Biotech presented three posters on peptide deformylase profiling the inhibitor, BB-3497 (**Figure 14**). The first poster by W Thomas *et al.* (British Biotech, Oxford, UK) presented a method of screening a library of metalloprotease inhibitors against the *E. coli* PDF. A novel high-throughput assay was developed using a fluorometric assay, utilising flourescamine as the amine reactive reagent. BB-3497 has excellent enzymatic activity against PDF of several organisms, *E. coli* 7 nM, *S. aureus* 100 nM and *S. pneumoniae* 30 nM. BB-3497 was shown to be selective for PDF when tested against a panel of mammalian metalloproteases. JM Clements *et al.*, (British Biotech) presented a poster on antibacterial activity of BB-3497 against a panel of Gram-positive and Gram-negative organisms showed moderate activity ranging from 0.25 - 32 μg/ml. Mechanism of action studies used an *E. coli* strain with the deletion of *fmt*, removing the need for deformylation and an *E. coli* strain with PDF on a plasmid for overexpression. The whole cell activity of BB-3497 against the *E. coli* parent strain and the mutant *E. coli* had MIC values of 4 and > 128 μg/ml, respectively, showing specificity of the compound for deformylase by the increased MIC in the mutant strain. The bactericidal effects were evaluated for BB-3497 by *in vitro* time-kill studies against *S. aureus*, *E. coli* and *E. faecalis* at concentrations 4 times MIC. For all organisms, the decrease in viable counts was less than one log, which showed bacteriostatic activity. Rate of resistance emergence was determined for BB-3497 at 4 times MIC against *E. coli*, *S. aureus*, *E. faecalis*, *S. pneumoniae* and *H. influenzae*. The rates were 10^{-7} for *E. coli*, *S. aureus* and *E. faecalis*, while the rates were much lower at 10^{-9} for *S. pneumoniae* and *H. influenzae*. Mutations in the resistant strains of *E. coli*, *S. aureus* and *E. faecalis* were found to be either missense or frameshifts in the *fmt* gene. Pharmacokinetics of BB-3497 given iv. or po. at a dose of 100 mg/kg was rapidly absorbed in mice which showed a C_{max} of 23 mg/l and a $AUC_{0\text{-}24}$ of 23 mg.h/l. *In vivo* efficacy was tested in a murine systemic infection of *S. aureus* with

a single iv. or po. dose of BB-3497 given 1 h following infection. An ED_{50} of 7 mg/kg, iv. dose and 8 mg/kg, po. dose, for *S. aureus* (Smith strain) and 14 mg/kg, p.o. dose, for methicillin resistant *S. aureus*. P Baker *et al*. (British Biotech and Krebs Institute for Biomolecular Research, University of Sheffield, UK) presented data on x-ray crystal structures of actinonin and BB-3497, which showed similar binding in the complex, partially within the active site. The most suitable sites for modification of compounds to improve antibacterial activity appears to be P2′ and P3′.

13. Summary and expert opinion

For the first time in many years, the ICAAC meeting was rich, with a variety of late-stage development antimicrobial compounds, filling multiple unmet medical needs, with a high probability of reaching the marketplace. Novel anti-MRSA carbapenems, novel quinolones, new cephalosporins, the novel class ketolides and novel antifungal agents top this list of new agents. The innovative approaches to adjunct therapy (novel β-lactamase inhibitors, efflux pump inhibitor), as well as long-term genomics efforts, offer great excitement and hope to combat emerging resistance problems in the clinic.

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