Comparative Antimicrobial Characterization of LBM415 (NVP PDF-713), A New Peptide Deformylase Inhibitor of Clinical Importance

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ABSTRACT

Background: LBM415 (415) is a leading member of the PDI class being developed for clinical trials for treatment of community-acquired respiratory and skin and soft tissue infections. This study presents results of microbiologic studies characterizing 415.
Methods: 1,306 recent (2001-2002) clinical isolates were selected to over-represent resistant (R) populations. MIC, MBC and spontaneous mutation rates were determined using NCCLS reference agar and broth microdilution methods and included use of efflux inhibitors (reserpine, phe-arg-ß-naphthylamide [PABN]) when testing *H. influenzae* (HI).
Results: All staphylococci (153 strains; MIC_{90}, 2 µg/ml), streptococci (320 strains; MIC_{90}, 0.5 - 1 µg/ml), enterococci (104 strains; MIC_{90}, 4 µg/ml), *Moraxella catarrhalis* (103 strains; MIC_{90}, 0.5 µg/ml) and *Legionella pneumophila* (50 strains; MIC_{90}, 0.12 µg/ml) were inhibited at ≤ 8 µg/ml of 415, as were 97% of HI (300 strains; MIC_{90}, 4 – 8 µg/ml). 100% of Gram-positive and –negative anaerobes (31 strains; MIC_{90}, 1 µg/ml) were inhibited by ≤ 4 µg/ml, whereas Enterobacteriaceae (112 strains) and most non-fermentative bacilli (107 strains) were not inhibited at readily achievable concentrations of 415. The new PDI had a dominantly bacteriostatic action, and spontaneous single step mutational rates occurred at low levels (10^{-7} to $< 10^{-9}$). Neither class-specific synergistic nor antagonistic interactions were observed. Whereas agar-based MIC results trended towards a two-fold higher MIC than the broth method and was inoculum dependent, other variations in incubation environment, media supplements, pH or calcium concentration had little influence on 415 MICs. Use of PABN showed an average of one log, dilution decrease in HI MICs, demonstrating the contribution of efflux pumps to R.
Conclusions: The in vitro activity of 415 against targeted bacterial species, including R subsets, and other laboratory characteristics of this PDI demonstrate the potential of the class as novel antimicrobial agents.

INTRODUCTION

Peptide deformylase, a highly conserved metalloproteinase, has been observed to be critical to the maturation of proteins during translation in prokaryotic cells and is the target for a new class of antimicrobial agent, the peptide deformylase inhibitors (PDI). LBM415 (NVP PDF-713) is a *N*-alkyl urea hydroxamic acid that is the first of the PDI class to advance to clinical trials for the oral and parenteral treatment of respiratory tract, and skin and skin structure infections caused by susceptible Gram-positive and -negative organisms (Figure 1).

In this report we summarize the initial results of testing LBM415 and selected comparator agents against a world-wide collection of contemporary, clinical isolates chosen to over-represent current resistance trends. Additional LBM415 microbiologic features examined included the determination of bactericidal concentrations, rate of bacterial killing, and interactions (synergy testing) with other antimicrobials. Lastly, single step mutational rates were determined to assess the ability of various bacterial species to develop resistance to a PDI.

MATERIALS AND METHODS

- Bacterial strains. A total of 1,306 recent (2001-early 2002) clinical strains were selected and included: 153 staphylococci (90 oxacillin-resistant), 170 *Streptococcus pneumoniae* (65, 52 and 53 penicillin-susceptible, -intermediate and -resistant, respectively), 69 ß-haemolytic streptococci (42 erythromycin-resistant), 81 viridans group *Streptococcus* spp. (only 40.7% susceptible to penicillin), 74 vancomycin-susceptible enterococci, 30 vancomycin-resistant enterococci (only 53.3% susceptible to quinupristin/dalfopristin), 300 *Haemophilus influenzae* (130 ampicillin-resistant), 103 *Moraxella catarrhalis* (90.3% penicillin-resistant), 112 Enterobacteriaceae (14 species or genus groups), 107 non-fermentative gram-negative bacilli (six species or genus groups), 31 anaerobes, 50 *Legionella pneumophila* and 26 other Gram-positive cocci.
- Antimicrobial susceptibility testing. Compound LBM415 was obtained from Novartis Pharmaceuticals (Summit, NJ, USA). Comparison agents were provided by the manufacturers in the USA of each agent or by Sigma Chemicals (St. Louis, MO, USA). All MIC and MBC tests were performed using NCCLS reference broth microdilution methods and interpretive criteria. Agar dilution was used for testing of anaerobes (Brucella blood agar) and *L. pneumophila* (buffered yeast extract agar with and without charcoal).
- Kill-curve experiments used initial inoculum densities of 5 x 10^7 CFU/ml and drug concentrations at 2X, 4X and 8X MIC. Drug interaction (synergy) tests used co-drugs at concentrations of MIC/4 combined with LBM415 at 2X, 4X and 8X MIC. Synergy was defined as a ≥ two log_{10} CFU/ml decrease in the inoculum at any monitored time compared to the activity of LBM415 alone (4X MIC).
- *H. influenzae* were tested in HTM broth for LBM415 alone and with two efflux inhibitors (reserpine and phe-arg-8-napththylamide) at concentrations of 10 and 20 µg/ml. Five strains were compared against both inhibitors and 20 additional strains were screened for LBM415 combined with only phe-arg-8-naphthylamide.
- The effects of changing standardized susceptibility testing conditions (inoculum, environment, media, pH, and divalent cation concentration) on the LBM415 MIC results were assessed by reference agar dilution methods and compared to broth microdilution test results.
- Spontaneous mutation rates and passaging studies. Single-step mutational rates to resistance were determined by plating a 10^9 CFU organism suspension (20 strains) onto MH agar plates containing LBM415 at concentrations 2X, 4X and 8X the established MIC. The frequency of mutation was determined by colony counts at 24 and 48 hours.

RESULTS

- All LBM415 MIC values were ≤ 2 µg/ml for *S. aureus*, with $MIC_{50/90}$ values of 1/2 µg/ml and 0.5/2 µg/ml for oxacillin-susceptible and oxacillin resistant strains, respectively (Table 1).
- Coagulase-negative staphylococcal strains were slightly less susceptible to LBM415 than were *S. aureus*, with MIC ranges extending to 2 µg/ml for oxacillin-susceptible strains and to 4 µg/ml for oxacillin-resistant strains.
- Among all *S. pneumoniae* strains, LBM415 was uniformly active with MIC_{50} results of 0.5 to 1 µg/ml. All strains were uniformly susceptible to levofloxacin, vancomycin and quinupristin/dalfopristin (100%) with increased resistance documented to ceftriaxone, erythromycin, clindamycin, and chloramphenicol among penicillin non-susceptible strains.
- LBM415 was uniformly active against ß-haemolytic and viridans group streptococci (MIC_{90} values 1 and 0.5 µg/ml, respectively); viridans group strains displayed variable resistance to all comparators except for linezolid and vancomycin (100% susceptible).
- No difference was noted in the activity of LBM415 against vancomycin susceptible or resistant enterococci.
- LBM415 displayed activity against both *H. influenzae* ($MIC_{50/90}$ of ampicillin-susceptible and ampicillin-resistant strains being 1/4 µg/ml, and 2/8 µg/ml, respectively) and *M. catarrhalis* (0.25/0.5 µg/ml, respectively; Table 2). All *H. influenzae* and *M. catarrhalis* strains remained highly susceptible to the comparator agents used clinically.
- The activity of LBM415 against *L. pneumophilia* was equal to that of levofloxacin (MIC_{90}, 0.12 µg/ml) and eight-fold more potent than erythromycin. The use of charcoal in the BYE test medium markedly reduced LBM415 activity (data not shown).
- The cumulative percentage of bacterial strains inhibited at specific MIC values is displayed in Table 3. Among the most common bacterial pathogens of community-acquired pneumonia and skin and skin structure infections, all staphylococci, streptococci, enterococci, *M. catarrhalis* and *L. pneumophila* are inhibited at 8 µg/ml or less of LBM415. *H. influenzae* strains were only slightly less susceptible (97%) to LBM415.
- Enterobacteriaceae and most non-fermentative gram-negative bacilli were not inhibited by LBM415 achievable serum concentrations.
- Variations in broth and agar MIC test conditions demonstrated that, whereas the agar-based method trended towards a one log_2 dilution higher MIC than the broth method and was inoculum dependent, other variations in incubation environment, media supplements, pH or calcium concentration had little influence on LBM415 MIC results (data not shown).
- Whereas the use of reserpine demonstrated no significant MIC reduction in *H. influenzae*, phe-arg-ß-naphthylamide showed an average of one log_2 dilution decrease in *H. influenzae* MICs, demonstrating the contribution of efflux pumps (data not shown).
- The results of the single-step mutation tests at 8X MIC of LBM415 demonstrated that, generally, mutation rates were extremely low for enterococci, *S. pyogenes*, *S. pneumoniae*, *M. catarrhalis*, *H. influenzae* and some strains of *S. aureus*. Higher mutational frequencies were noted for single strains of oxacillin-susceptible or -resistant *S. aureus* and CoNS.
- Table 4 summarizes the results that demonstrate a dominant bacteriostatic action for this new PDF inhibitor when using kill-curve methods. Bactericidal action at 24 hours, usually at concentrations 4X or 8X MIC, was observed with four strains (20%; oxacillin-resistant *S. aureus*, oxacillin-susceptible CoNS, penicillin-resistant *S. pneumoniae*, and *H. influenzae*).
- Drug interaction (synergy) results were derived from 28 kill curve tests for 14 organisms. Only two studies showed synergy (LBM415 + gentamicin versus *S. mitis*; LBM415 + ampicillin versus vancomycin-resistant *E. faecalis*). All other interactions were classified as indifferent and no antagonism was observed (data not shown).

TABLE 1. Comparative activity screen of LBM415, a deformylase inhibitor, and selected comparison classes of antimicrobial agents tested against staphylococci (153 strains), streptococci (320 strains) and enterococci (130 strains).

Antimicrobial agent (no. tested)	MIC (µg/ml) 50%	90%	Range	% susceptible[a]	% resistant[a]
S. aureus					
oxacillin-susceptible (53)					
LBM415	1	2	0.12-2	-[b]	-[b]
Linezolid	2	2	0.5-2	100.0	0.0
Quinupristin/Dalfopristin	0.25	0.5	0.12-0.5	100.0	0.0
Vancomycin	1	1	0.5-2	100.0	0.0
oxacillin-resistant (51)					
LBM415	0.5	2	0.25-2	-[b]	-[b]
Linezolid	2	2	1-2	100.0	0.0
Quinupristin/Dalfopristin	0.5	0.5	0.12-2	98.0	0.0
Vancomycin	1	2	0.5-4	100.0	0.0
Coagulase-neg. staphylococci					
oxacillin-susceptible (10)					
LBM415	1	2	0.25-2	-[b]	-[b]
Linezolid	1	1	0.5-1	100.0	0.0
Quinupristin/Dalfopristin	0.12	0.25	0.12-0.25	100.0	0.0
Vancomycin	1	2	1-2	100.0	0.0
oxacillin-resistant (39)					
LBM415	1	2	0.25-4	-[b]	-[b]
Linezolid	1	1	0.5-2	100.0	0.0
Quinupristin/Dalfopristin	0.25	0.25	≤0.06-0.25	100.0	0.0
Vancomycin	2	2	1-2	100.0	0.0
S. pneumoniae					
penicillin-susceptible (65)					
LBM415	0.5	1	0.03-2	-[b]	-[b]
Ceftriaxone	0.015	0.03	≤0.008-0.06	100.0	0.0
Erythromycin	0.25	4	≤0.25->32	86.2	13.8
Quinupristin/Dalfopristin	0.5	0.5	0.12-0.5	100.0	0.0
Levofloxacin	1	1	0.5-2	100.0	0.0
penicillin-intermediate (52)					
LBM415	0.5	1	≤0.016-1	-[b]	-[b]
Ceftriaxone	0.25	0.5	0.03-1	100.0	0.0
Erythromycin	2	>32	≤0.25->32	46.2	51.9
Quinupristin/Dalfopristin	0.5	0.5	0.25-1	100.0	0.0
Levofloxacin	1	1	0.5-1	100.0	0.0
penicillin-resistant (53)					
LBM415	0.25	0.5	0.06-1	-[b]	-[b]
Ceftriaxone	1	2	≤0.008-8	69.8	3.8
Erythromycin	2	>32	≤0.25->32	43.4	54.7
Quinupristin/Dalfopristin	0.5	0.5	0.25-1	100.0	0.0
Levofloxacin	1	1	0.5-1	100.0	0.0
ß-haemolytic streptococci (69)					
LBM415	0.5	1	0.06-4	-[b]	-[b]
Penicillin	≤0.015	0.06	≤0.016-0.12	100.0	0.0
Erythromycin	≤0.06	8	≤0.06->8	85.5	14.5
Linezolid	1	1	≤0.06-1	100.0	0.0
Levofloxacin	0.5	1	0.12-2	100.0	0.0
viridans group streptococci (81)					
LBM415	0.25	0.5	0.03-4	-[b]	-[b]
Penicillin	0.5	4	≤0.016->32	40.7	22.2
Erythromycin	0.12	>8	≤0.06->8	51.9	42.0
Linezolid	1	1	0.12-2	100.0	0.0
Levofloxacin	1	2	≤0.03->4	93.8	4.9
Enterococci[c]					
vancomycin-susceptible (74)					
LBM415	2	4	0.06-8	-[b]	-[b]
Ampicillin	≤2	>16	≤2->16	86.5	13.5
Chloramphenicol	8	>16	≤2->16	82.4	14.9
Quinupristin/Dalfopristin	8	8	0.25->8	14.9	66.2
Linezolid	2	2	0.5-8	97.3	2.7
vancomycin-resistant (30)					
LBM415	2	4	0.25-8	-[b]	-[b]
Ampicillin	>16	>16	≤2->16	33.3	66.7
Chloramphenicol	8	>16	≤2->16	63.3	26.7
Quinupristin/Dalfopristin	1	>8	0.25->8	53.3	40.0
Linezolid	2	2	1->8	93.3	6.6
Other Gram-pos. species (26)[d]					
LBM415	0.25	8	0.03->32	-[b]	-[b]

a. Susceptibility criteria of the NCCLS [2004].
b. No criteria have been established.
c. Isolates include *Enterococcus avium* (one strain), *E. casseliflavus* (three strains), *E. durans* (two strains), *E. faecalis* (62 strains), *E. faecium* (32 strains), *E. gallinarum* (three strains), and *E. hirae* (one strain).
d. Isolates include *Aerococcus* spp. (two strains), *Bacillus* spp. (three strains), *Corynebacterium* spp. (six strains), *Gemella* spp. (one strain), *Lactobacillus* spp. (two strains), *Lactococcus* spp. (one strain), *Leuconostoc* spp. (two strains), *Listeria* spp. (three strains), *Micrococcus* spp. (three strains), *Nocardia* spp. (two strains), and *Stomatococcus* spp. (one strain).

TABLE 2. Comparative antimicrobial activity screen of LBM415, a deformylase inhibitor, tested against *H. influenzae* (300 strains) and *M. catarrhalis* (103 strains).

Antimicrobial agent (no. tested)	MIC (µg/ml) 50%	90%	Range	% susceptible[a]	% resistant[a]
H. influenzae					
ampicillin-susceptible (170)					
LBM415	1	4	0.03-16	-[b]	-[b]
Amoxicillin/Clavulanate	0.5	2	≤0.06-4	100.0	0.0
Rifampin	≤1	≤1	≤1->2	99.2	0.8
Levofloxacin	≤0.03	≤0.03	≤0.03-0.06	100.0	-[b]
Azithromycin	1	2	≤0.12-4	100.0	0.0
ampicillin-resistant (130)					
LBM415	2	8	0.5-32	-[b]	-[b]
Amoxicillin/Clavulanate	≤2	≤2	≤2-8	98.3	1.7
Ceftriaxone	≤0.25	≤0.25	≤0.25	100.0	-[b]
Levofloxacin	≤0.03	≤0.03	≤0.03-0.25	100.0	-[b]
Azithromycin	1	2	0.25->16	99.2	0.8
***M. catarrhalis* (103)**					
LBM415	0.25	0.5	0.03-0.5	-[b]	-[b]
Penicillin	2	>4	≤0.03->4	9.7[c]	90.3[c]
Amoxicillin/Clavulanate	≤0.06	0.25	≤0.06-0.5	100.0	0.0
Azithromycin	≤0.12	≤0.12	≤0.12	100.0	0.0
Tetracycline	≤2	≤2	≤2	100.0	0.0

a. Susceptibility criteria of the NCCLS [2004]. Breakpoints for *H. influenzae* were applied for interpretation of *M. catarrhalis* results.
b. No criteria have been established.
c. Susceptibility predicted by a negative ß-lactamase test result (MIC, ≤ 0.06 µg/ml).

TABLE 3. Summary of antimicrobial activity at individual MIC values of LBM415 tested against 11 organism groups.

Organisms (no. tested)	Cumulative % inhibited at MIC (µg/ml): ≤0.25	0.5	1	2	4	8	16	32
Gram-positive								
S. aureus (104)	31	52	79	100	-	-	-	-
Coagulase-negative staphylococci (49)	12	39	69	98	100	-	-	-
S. pneumoniae (170)	52	87	99	100	-	-	-	-
Streptococci, not *S. pneumoniae* (150)	59	76	95	99	100	-	-	-
Enterococci (104)	12	27	43	65	95	100	-	-
Other Gram-positive species (26)	58	58	73	81	89	92	92	96
Gram-negative								
H. influenzae (300)	2	9	41	74	88	97	99	100
M. catarrhalis (103)	89	100	-	-	-	-	-	-
Enterobacteriaceae (112)[a]	0	0	0	0	0	0	0	5
Non-fermentative bacilli (107)[b]	0	0	0	1	2	5	11	33
Gram-positive and -negative								
Anaerobes (31)[c]	81	87	90	94	100	-	-	-

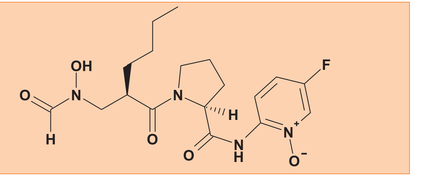
a. Includes *C. freundii* (two strains), *Citrobacter* spp. (two strains), *E. aerogenes* (two strains), *E. cloacae* (13 strains), *Enterobacter* spp. (seven strains), *E. coli* (40 strains), *K. oxytoca* (one strain), *K. ozaenae* (one strain), *K. pneumoniae* (21 strains), *Klebsiella* spp. (one strain), *M. morganii* (one strain), *P. mirabilis* (seven strains), *Salmonella* spp. (six strains) and *Serratia* spp. (eight strains).
b. Includes *Acinetobacter* spp. (22 strains), *B. cepacia* (four strains), *C. luteola* (two strains), *P. aeruginosa* (72 strains), *P. fluorescens* (three strains) and *S. maltophilia* (four strains).
c. Includes *Bacteroides fragilis* (13 strains), *B. fragilis* group species (four species; seven strains), *Clostridium* spp. (four species; nine strains) and *Fusobacterium* spp. (two species; two strains).

TABLE 4. Time-kill analysis of 20 organisms tested against LBM415 at three concentrations (2X, 4X and 8X MIC).

Organism (no. tested)/MIC level	No. of strains killed at time and log_{10} variation: 4 hours -1	-2	-3	8 hours -1	-2	-3	24 hours -1	-2	-3
***S. aureus* (4)[a]**									
2X MIC	0	0	0	0	0	0	3	3	0
4X MIC	0	0	0	0	0	0	4	3	1
8X MIC	0	0	0	0	0	0	4	3	1
CoNS (4)[a]									
2X MIC	0	0	0	0	0	0	2	2	1
4X MIC	0	0	0	0	0	0	3	2	1
8X MIC	0	0	0	0	0	0	3	2	1
Enterococci (4)[c]									
2X MIC	0	0	0	1	1	0	1	1	0
4X MIC	1	0	0	1	1	0	0	0	0
8X MIC	1	1	0	1	1	0	1	0	0
Streptococci (6)[d]									
2X MIC	0	0	0	0	0	0	2	0	0
4X MIC	0	0	0	0	0	0	4	2	0
8X MIC	0	0	0	0	0	0	5	2	1
***M. catarrhalis* (1)**									
2X MIC	0	0	0	0	0	0	0	0	0
4X MIC	0	0	0	0	0	0	1	0	0
8X MIC	0	0	0	0	0	0	1	0	0
***H. influenzae* (1)**									
2X MIC	0	0	0	1	0	0	0	0	0
4X MIC	1	0	0	1	1	1	1	1	1
8X MIC	1	0	0	1	1	1	1	1	1

a. Two strains were oxacillin-resistant.
b. CoNS = coagulase-negative staphylococci; two strains were oxacillin-resistant.
c. Two *E. faecalis* (one vancomycin-resistant) and two *E. faecium* (one vancomycin-resistant).
d. Two viridans group streptococci, one *S. pyogenes*, one *S. agalactiae*, and two *S. pneumoniae* (one penicillin-resistant) strains.

FIGURE 1. Chemical structure of LBM415, a novel peptide deformylase inhibitor.



CONCLUSIONS

- PDF inhibitors are a novel class of antimicrobic with bacteriostatic properties, rapidly being developed for use as a therapeutic alternative for agents used in the outpatient arena in the treatment of community-acquired respiratory tract infections and for infections of skin and skin structures.
- The spectrum of activity and potency for use against the targeted bacterial species appears to be adequate.
- The development of resistance can be expected to occur no more rapidly than for other indicated antimicrobial agents with similar clinical indications.
- LBM415 will be the first PDF inhibitor to be introduced for clinical trial use and promises to be a significant advance in therapeutics should the pharmacokinetic/pharmacodynamic parameters in humans support continued, expanded development.

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