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Antimicrobial Activity of the Investigational Pleuromutilin BC-3781 Against Organisms Responsible for Community-Acquired Respiratory Tract Infections (CA-RTI)

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ABSTRACT

Background: BC-3781 is an investigational semi-synthetic pleuromutilin derivative, which inhibits ribosomal protein synthesis. BC-3781 binds to the 50S ribosomal subunit and cross resistance (R) with other antimicrobial classes is uncommon. We evaluated the activity of BC-3781 against CA-RTI pathogens.

Methods: BC-3781 and comparator agents were susceptibility tested against *S. pneumoniae* (SPN; 157 isolates; 33% penicillin [PEN]-R), *H. influenzae* (HI; 102; 50% β-lactamase [BL]-producers), *M. catarrhalis* (MCAT; 50) and *L. pneumophila* (LP; 30) by CLSI broth microdilution (BMD) method. *M. pneumoniae* (MP; 4 strains) was tested by BMD and agar dilution methods, while *Chlamydophila pneumoniae* (CP; 2 strains) MIC values were determined on monolayers of HEp-2 cells.

Results: BC-3781 was eight- and 16-fold more active than azithromycin (AZI) and levofloxacin against SPN, respectively; and its activity was not adversely affected by PEN-R. SPN showed high R rates to AZI (50.3%) and clindamycin (31.2%). HI and MCAT exhibited low BC-3781 MIC values independent of BL production. BC-3781 activity against LP was similar to that of erythromycin but lower than AZI. BC-3781 MIC ranges for MP and CP were ≤0.0003-0.0006 and 0.01-0.04 µg/mL, respectively.

Conclusions: BC-3781 was very active (MIC_{90}, 0.25-2 µg/mL) against organisms commonly associated with CA-RTI and not negatively influenced by R to other antimicrobials. Further studies appear warranted to define the role of this novel pleuromutilin for the treatment of CA-RTI.

Organism (no. tested)	BC-3781		Azithromycin		Levofloxacin	
	$MIC_{50/90}$[a]	Range[a]	$MIC_{50/90}$[a]	Range[a]	$MIC_{50/90}$[a]	Range[a]
SPN (157)	0.12/0.25	0.015-0.5	2/>16	0.015->16	1/1	0.25-16
HI (102)	0.5/2	0.25-2	1/2	≤0.5-4	≤0.06/≤0.06	≤0.06-0.12
MCAT (50)	0.06/0.12	0.015-0.12	≤0.5/≤0.5	≤0.5	≤0.06/≤0.06	≤0.06-1
LP (30)[b]	0.06/0.5	0.06-1	0.015/0.015	0.0004-0.03	0.015/0.015	0.007-0.03

[a] MIC values in µg/mL; [b] Results of BMD method using buffer yeast extract medium (no charcoal with α–ketoglutarate).

INTRODUCTION

Community-acquired respiratory tract infections (CA-RTI), especially pneumonia (CABP), represent the main causes of morbidity and mortality among children and adults. The dominant bacterial causes of CA-RTI are *Streptococcus pneumoniae* and *Haemophilus influenzae*. Furthermore, a significant proportion of CABP cases are caused by the "atypical agents", mainly *Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Legionella pneumophila.* Thus, it has been recommended that empiric antimicrobial therapy for severe CA-RTI should provide antimicrobial coverage for these organisms, including multidrug-resistant (MDR) *S. pneumoniae,* β-lactamase-producing *H. influenzae* and, in some geographic regions, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).

BC-3781, a semi-synthetic pleuromutilin derivative, is currently in clinical development for both oral and intravenous administration. Results from human phase I clinical trials have demonstrated that BC-3781 can achieve therapeutically relevant blood and tissue levels with excellent tolerability when administered by either route of administration. BC-3781 acts by binding to a unique site of the peptidyl transferase component of the 50S subunit of ribosomes and cross-resistance to other antimicrobial classes is uncommon. We evaluated the activity of BC-3781 against CA-RTI pathogens.

MATERIALS & METHODS

Bacterial isolates: The organism collection evaluated in the present study included 157 *S. pneumoniae* (33% penicillin-resistant), 102 *H. influenzae* (50% β-lactamase-producers) and 50 *M. catarrhalis* collected from patients with CA-RTI from medical centers located in the USA and various European countries. In addition, 30 clinical *L. pneumophila* isolates of five different serogroups from Germany (2007-2008), four *M. pneumoniae* (ATCC 15531, ATCC 15293, ATCC 49894, ATCC 29342) and two *C. pneumoniae* strains (ATCC VR-1310, ATCC VR-1360) were also included.

Susceptibility testing: *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* were tested for susceptibility to BC-3781 and various comparator agents by broth microdilution methods following the Clinical and Laboratory Standards Institute (CLSI) recommendations (M07-A8, 2009). 96-well frozen-form assay panels were produced by JMI Laboratories and consisted of three media types, cation-adjusted Mueller-Hinton broth, cation-adjusted Mueller-Hinton broth with 3-5% lysed horse blood (for testing of streptococci) and Haemophilus Test Medium (for testing of *H. influenzae)*. Inoculums were prepared by making direct broth suspensions of isolated colonies selected from an 18- to 24-hour agar plate. The broth suspensions were adjusted, using a photometric device, to achieve a turbidity equivalent of a 0.5 McFarland standard (approximately 1 to $2x10^8$ CFU/ml). These inoculum preparations were diluted to achieve a final concentration of approximately 5×10^5 CFU/ml and used to inoculate the wells of the MIC panels. Concurrent testing of quality control (QC) strains determined that proper test conditions were applied. These strains included: *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247 and *S. aureus* ATCC 29213.

Susceptibility of *L. pneumophila* was determined by broth microdilution in buffered yeast extract medium supplemented with 0.1% α-ketoglutarate (BYEα) using an inoculum of $3x10^5$ CFU/ml. Additionally MICs were determined by agar dilution technique using BYCEα medium containing 25% charcoal and an inoculum size of 10^4-10^5 CFU/spot. MICs were read three days after incubation at 35°C in humidified atmosphere. *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 served as controls.

Susceptibility of *M. pneumoniae* was determined by broth microdilution in SP-4 medium (pH 7.6) supplemented with CMRL 1066 medium, 200 mM L-glutamine, yeast extract, yeastolate, inactivated fetal calf serum and phenol red using an inoculum size of 0.5 x 10^4-10^5 CCU/ml as described by Hannan (2000) and Ridgway (2001).

C. pneumoniae testing was performed on HEp-2 monolayers seeded on glass coverslips in 24-well plates. HEp-2 cells infected with *C. pneumoniae* (final inoculum 10^3 -10^4 IFU/ml) were treated with the test compounds dissolved in IMDM medium supplemented with L-glutamine, phenol red, HEPES, sodium hydrogen carbonate, fetal calf serum, MEM vitamins, non essential amino acids, glucose and cycloheximide at 35°C in humidified atmosphere with 5% CO_2 for 72 h.

C. pneumoniae inclusions were then stained using the immunofluorescence monoclonal antibody (Pathfinder Chlamydia Culture Confirmation System, Biorad, Austria). MICs were defined as the lowest concentration of antibiotic at which no inclusions were observed.

RESULTS

- All *S. pneumoniae* were inhibited by ≤0.5 µg/mL BC-3871 and the compound was comparably potent against penicillin-susceptible (MIC_{90}, 0.25 µg/mL), -intermediate (MIC_{90}, 0.12 µg/mL) and -resistant (MIC_{90}, 0.25 µg/mL) isolates (Table 1). BC-3781 was the most potent agent tested against this *S. pneumoniae* collection with MIC_{50} of 0.12 µg/mL and MIC_{90} of 0.25 µg/mL (Table 2).
- Resistance to macrolides, clindamycin and trimethoprim/sulfamethoxazole among *S. pneumoniae* increased considerably, as the penicillin MIC values increased. Among the penicillin-resistant (MIC ≥2 µg/mL) isolates, macrolide resistance was 75.0% and over 50% of the isolates were resistant to clindamycin. Less than 2% of the tested *S. pneumoniae* isolates were non-susceptible to levofloxacin and all strains were susceptible to vancomycin and linezolid.
- BC-3781 (MIC_{50}, 0.12 µg/mL and MIC_{90}, 0.25 µg/mL) was eight- to 16-fold more active than azithromycin (MIC_{50}, 2 µg/mL and MIC_{90}, >16 µg/mL) and levofloxacin (MIC_{50}, 1 µg/mL and MIC_{90}, 1 µg/mL) against *S. pneumoniae* (Table 1).

Table 1. MIC frequency distributions of investigational Nabriva agent BC-3781 tested against bacterial isolates from respiratory tract infections

Organism (no. tested)	Cumulative percentage of strains inhibited at each MIC (µg/mL)							
	0.015	0.03	0.06	0.12	0.25	0.5	1	2
S. pneumoniae (157)								
Penicillin-susceptible (54)	3.7	7.4	24.1	61.1	100.0	-	-	-
Penicillin-intermediate (51)	3.9	13.7	58.8	94.1	100.0	-	-	-
Penicillin-resistant (52)	1.9	1.9	21.2	88.5	98.1	100.0	-	-
H. influenzae (102)	-	-	-	-	7.8	56.9	87.3	100.0
Beta-lactamase negative (51)	-	-	-	-	7.8	56.9	92.2	100.0
Beta-lactamase positive (51)	-	-	-	-	7.8	56.9	82.4	100.0
M. catarrhalis (50)	2.0	4.0	74.0	100.0	-	-	-	-

RESULTS

Table 2. *In vitro* activity of BC-3781 in comparison to selected antimicrobial agents tested against bacterial strains from respiratory infections

Antimicrobial agent	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC Range (µg/mL)	% susceptible/ resistant[a]
S. pneumoniae (157)				
BC-3781	0.12	0.25	0.015–0.5	- / -
Penicillinb	0.25	>2	≤0.03–>2	87.3 / 0.0
Penicillinc	0.25	>2	≤0.03–>2	34.4 / 33.1
Erythromycin	0.5	>16	0.015–>16	49.7 / 49.7
Clindamycin	0.06	>16	≤0.008–>16	68.2 / 31.2
Azithromycin	2	>16	0.015–>16	49.7 / 50.3
Doxycycline	0.12	8	0.03–16	- / -
Levofloxacin	1	1	0.25–16	98.1 / 1.3
Vancomycin	0.5	0.5	≤0.12–0.5	100.0 / -
Linezolid	1	1	≤0.25–2	100.0 / -
Trimethoprim/ sulfamethoxazole	1	8	≤0.5–>8	43.9 / 43.3
Penicillin-susceptible (MIC, ≤0.06 µg/ml; 54)				
BC-3781	0.12	0.25	0.015–0.25	- / -
Erythromycin	0.06	4	0.015–>16	83.3 / 14.8
Clindamycin	0.06	0.06	≤0.008–>16	94.4 / 5.6
Azithromycin	0.12	16	0.015–>16	83.3 / 16.7
Doxycycline	0.12	4	0.03–8	- / -
Levofloxacin	1	1	0.5–4	98.1 / 0.0
Vancomycin	0.5	0.5	≤0.12–0.5	100.0 / -
Linezolid	1	1	≤0.25–1	100.0 / -
Trimethoprim/ sulfamethoxazole	≤0.5	2	≤0.5–>8	83.3 / 7.4
Penicillin-intermediate (MIC, 0.12-1 µg/ml; 51)				
BC-3781	0.06	0.12	0.015–0.25	- / -
Erythromycin	4	>16	0.015–>16	39.2 / 60.8
Clindamycin	0.06	>16	0.015–>16	60.8 / 37.3
Azithromycin	4	>16	0.03–>16	39.2 / 60.8
Doxycycline	0.12	16	0.06–16	- / -
Levofloxacin	1	1	0.5–8	98.0 / 2.0
Vancomycin	0.25	0.5	≤0.12–0.5	100.0 / -
Linezolid	1	1	0.5–1	100.0 / -
Trimethoprim/ sulfamethoxazole	1	8	≤0.5–8	39.2 / 33.3
Penicillin-resistant (MIC, ≥2 µg/ml; 52)				
BC-3781	0.12	0.25	0.015–0.5	- / -
Erythromycin	>16	>16	0.03–>16	25.0 / 75.0
Clindamycin	>16	>16	0.03–>16	48.1 / 51.9
Azithromycin	>16	>16	0.06–>16	25.0 / 75.0
Doxycycline	4	8	0.06–16	- / -
Levofloxacin	1	1	0.25–16	98.1 / 1.9
Vancomycin	0.5	0.5	0.25–0.5	100.0 / -
Linezolid	1	1	0.5–2	100.0 / -
Trimethoprim/ sulfamethoxazole	8	>8	≤0.5–>8	7.7 / 90.4

Antimicrobial agent	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC Range (µg/mL)	% susceptible/ resistant[a]
H. influenzae (102)				
BC-3781	0.5	2	0.25–2	- / -
Ampicillin	0.5	>4	≤0.25–>4	52.0 / 39.2
Amoxicillin/clavulanate	0.5	2	≤0.25–4	100.0 / 0.0
Azithromycin	1	2	≤0.5–4	100.0 / -
Doxycycline	≤0.5	1	≤0.5–16	95.1 / 1.0[d]
Levofloxacin	≤0.06	≤0.06	≤0.06–0.12	100.0 / -
Cefdinir	0.25	0.5	0.03–1	100.0 / -
Cefuroxime	0.5	1	0.12–2	100.0 / 0.0
Trimethoprim/ sulfamethoxazole	≤0.25	4	≤0.25–>8	81.4 / 15.7
β-lactamase-negative (51)				
BC-3781	0.5	1	0.25–2	- / -
Ampicillin	≤0.25	0.5	≤0.25–1	100.0 / 0.0
Amoxicillin/clavulanate	0.5	1	≤0.25–2	100.0 / 0.0
Azithromycin	1	2	≤0.5–2	100.0 / -
Doxycycline	≤0.5	≤0.5	≤0.5–1	100.0 / 0.0[d]
Levofloxacin	≤0.06	≤0.06	≤0.06	100.0 / -
Cefdinir	0.25	0.5	0.03–1	100.0 / -
Cefuroxime	0.5	2	0.12–2	100.0 / 0.0
Trimethoprim/ sulfamethoxazole	≤0.25	2	≤0.25–8	86.3 / 9.8
β-lactamase-positive (51)				
BC-3781	0.5	2	0.25–2	- / -
Ampicillin	>4	>4	≤0.25–>4	3.9 / 78.4
Amoxicillin/clavulanate	1	2	0.5–4	100.0 / 0.0
Azithromycin	1	2	≤0.5–4	100.0 / -
Doxycycline	≤0.5	1	≤0.5–16	90.2 / 2.0[d]
Levofloxacin	≤0.06	≤0.06	≤0.06–0.12	100.0 / -
Cefdinir	0.25	0.25	0.06–0.5	100.0 / -
Cefuroxime	0.5	1	0.12–2	100.0 / 0.0
Trimethoprim/ sulfamethoxazole	≤0.25	4	≤0.25–>8	76.5 / 21.6
M. catarrhalis (50)[e]				
BC-3781	0.06	0.12	0.015–0.12	- / -
Ampicillin	≤0.25	4	≤0.25–>4	80.0 / 20.0[f]
Amoxicillin/clavulanate	≤0.25	≤0.25	≤0.25–0.5	100.0 / 0.0[d]
Azithromycin	≤0.5	≤0.5	≤0.5	100.0 / 0.0[d]
Doxycycline	≤0.5	≤0.5	≤0.5–1	100.0 / 0.0[d]
Levofloxacin	≤0.06	≤0.06	≤0.06–1	100.0 / 0.0[d]
Cefdinir	0.12	0.25	0.06–0.5	- / -
Cefuroxime	0.5	2	0.25–4	88.0 / 4.0[d]
Trimethoprim/ sulfamethoxazole	≤0.25	≤0.25	≤0.25–2	98.0 / 2.0[d]

[a] Criteria as published by the CLSI [2010], [b] Criteria as published by the CLSI [2010] for 'Penicillin parenteral (non-meningitis)', [c] Criteria as published by the CLSI [2010] for 'Penicillin (oral penicillin V)', [d] Criteria as published by the EUCAST [2010], [e] Included 40 β-lactamase-positive and 10 β-lactamase-negative isolates [f] Based on β-lactamase production.

- BC-3781 was also shown to have activity against the fastidious Gram-negative respiratory pathogens (Tables 1 and 2). BC-3781 was similarly active against β-lactamase-positive (MIC_{50}, 0.5 µg/mL and MIC_{90}, 1 µg/mL) and β-lactamase-negative *H. influenzae* isolates (MIC_{50}, 0.5 µg/mL and MIC_{90}, 2 µg/mL) with MIC distributions that were nearly identical (Table 1).
- BC-3781 was very active against *M. catarrhalis*, regardless of β-lactamase production, with a MIC_{90} value of 0.12 µg/mL (Table 2).
- BC-3781 activity against *Legionella pneumophila* (MIC_{50}, 0.03 µg/mL and MIC_{90}, 0.5 µg/mL) was similar to that of erythromycin (MIC_{50}, 0.03 µg/mL and MIC_{90}, 0.12 µg/mL), but less than azithromycin (MIC_{50} and MIC_{90}, 0.015 µg/mL) (Table 3).

Table 3. Antimicrobial activity of BC-3781 against *L. pneumophilia*

Antimicrobial agent	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC Range (µg/mL)
L. pneumophilia (30)[a]			
BC-3781	0.06 (0.12)	0.5 (0.5)	0.06-1 (1.12-1)
Azithromycin	0.015 (0.06)	0.015 (0.12)	0.0004-0.03 (0.06-0.5)
Erythromycin	0.03 (0.06)	0.12 (0.03-1)	0.03-1 (0.06-1)
Moxifloxacin	0.015 (0.06)	0.015 (0.12)	0.0018-0.003 (0.06-0.25)
Levofloxacin	0.015 (0.25)	0.015 (0.12)	0.007-0.003 (0.06-0.25)

[a] MICs determined by broth microdilution using BYEα medium; MICs in brackets show MICs determined in BCYEα medium supplemented with charcoal

- BC-3781 was highly active against *C. pneumoniae* with MIC ranging from ≤0.0003-0.0006 µg/mL being up to 16-fold more potent than the tested macrolides and doxycycline and 128-fold more active than moxifloxacin (Table 4).
- Against *M. pneumoniae* BC-3781 exhibited potent activity with an MIC range of ≤0.0003-0.0006 µg/mL. BC-3781 was significantly more active than doxycycline, ciprofloxacin, clindamycin and erythromycin and as active as azithromycin (Table 4).

Table 4. Antimicrobial activity of BC-3781 against *M. pneumoniae* and *C. pneumoniae*

Antimicrobial agent	MIC Range (µg/mL)	Antimicrobial agent	MIC Range (µg/mL)
C. pneumophilia (2)		*M. pneumoniae (4)*	
BC-3781	0.01-0.04	BC-3781	≤0.0003-0.0006
Azithromycin	0.08-0.16	Azithromycin	0.00015-0.0003
Clarithromycin	0.02-0.08	Erythromycin	0.0025-0.005
Erythromycin	0.04-0.16	Clindamycin	0.4-0.8
Moxifloxacin	0.32-1.28	Doxycycline	0.04-0.04
Doxycycline	0.04-0.08	Ciprofloxacin	0.2-0.8

CONCLUSIONS

- BC-3781 inhibited all *S. pneumoniae* at ≤0.5 µg/mL, with no difference noted between penicillin-susceptible and -resistant subsets.
- All *H. influenzae* isolates were inhibited by ≤2 µg/mL, irrespective of β-lactamase production. All *M. catarrhalis* (MICs, ≤0.12 µg/mL) were very susceptible to BC-3781.
- BC-3781 exhibited potent activity against atypicals such as *M. pneumoniae*, *C. pneumoniae* and *L. pneumophilia* being equally or significantly more active than antibiotics currently in use.
- BC-3781 was very active (MIC_{90}, 0.25-2 µg/mL) against organisms commonly associated with CA-RTI and not negatively influenced by resistance to other antimicrobials. Further studies appear warranted to define the role of this novel pleuromutilin for the treatment of CA-RTI.

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