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Antimicrobial activity of the novel pleuromutilin antibiotic BC-3781 against organisms responsible for community-acquired respiratory tract infections (CARTIs)

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Background: BC-3781 is an investigational semi-synthetic pleuromutilin antibiotic, which recently finished a clinical Phase 2 trial in acute bacterial skin and skin structure infections. BC-3781 binds to the 50S ribosomal subunit and cross-resistance with other antimicrobial classes is uncommon. We evaluated the activity of BC-3781 against organisms responsible for community-acquired respiratory tract infections (CARTIs).

Methods: BC-3781 and comparator agents were susceptibility tested against *Streptococcus pneumoniae* (157 isolates; 33% penicillin resistant), *Haemophilus influenzae* (102; 50% β-lactamase producers), *Moraxella catarrhalis* (50) and *Legionella pneumophila* (30) by broth microdilution and the agar dilution method. *Mycoplasma pneumoniae* (50 strains) was tested by broth microdilution, while *Chlamydophila pneumoniae* (50 strains) MIC values were determined using HEp-2 cells.

Results: Against *S. pneumoniae* ($MIC_{50/90}$ 0.12/0.25 mg/L) BC-3781 was 16- and 8-fold more active than azithromycin ($MIC_{50/90}$ 2/>16 mg/L) and levofloxacin ($MIC_{50/90}$ 1/1 mg/L), respectively, and its activity was not adversely affected by resistance to penicillin. *S. pneumoniae* showed high resistance rates to azithromycin (50.3%) and clindamycin (31.2%), all being inhibited by BC-3781 at concentrations ≤0.5 mg/L. *H. influenzae* and *M. catarrhalis* exhibited low BC-3781 MIC values independent of β-lactamase production. BC-3781 activity against *L. pneumophila* ($MIC_{50/90}$ 0.06/0.5 mg/L) was similar to that of erythromycin, but lower than that of azithromycin. BC-3781 also showed potent activity against *M. pneumoniae* and *C. pneumoniae*, with $MIC_{50/90}$ of 0.006/0.006 and 0.02/0.04 mg/L, respectively.

Conclusions: BC-3781 was very active against organisms commonly associated with CARTIs and its activity was not negatively influenced by resistance to other antimicrobials.

Keywords: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma* spp., anti-infective development, antimicrobial resistance

Introduction

Community-acquired respiratory tract infections (CARTIs), especially community-acquired bacterial pneumonia (CABP), represent one of the main causes of morbidity and mortality among children and adults. The dominant bacterial causes of CARTIs 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*.[1,2]

Thus, it has been recommended that empirical antimicrobial therapy for severe CARTIs should provide antimicrobial coverage for these organisms, including multidrug-resistant *S. pneumoniae*, β-lactamase-producing *H. influenzae* and, in some geographical regions, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).[1]

BC-3781, a semi-synthetic pleuromutilin derivative antibiotic, is currently in clinical development for both oral and intravenous treatment of acute bacterial skin and skin structure infections (ABSSSIs) and CABP.[3–6] BC-3781 exhibits excellent antibacterial activity against skin pathogens such as *S. aureus*, β-haemolytic streptococci, viridans streptococci and *Enterococcus faecium* as well as against respiratory pathogens.[7] BC-3781 activity against respiratory pathogens has been confirmed in various murine models of infections using *S. pneumoniae*, *H. influenzae* and *S. aureus* [methicillin-resistant *Staphylococcus aureus*

(MRSA) and CA-MRSA] as causative agents. In the models employed, the efficacy of BC-3781 was superior to that of the standard of care antibiotics.[8] Further, the drug showed good penetration into the epithelial lining fluid (ELF). High exposure levels of BC-3781 in the ELF and its broad-spectrum activity against respiratory pathogens strongly support its potential use in the treatment of bacterial respiratory tract infections.[7–9] Results from a series of human Phase 1 clinical trials and a Phase 2 clinical trial for the treatment of ABSSSI have demonstrated that BC-3781 can achieve therapeutically relevant blood and tissue levels with excellent tolerability when administered either orally or intravenously and that patients with ABSSSI can be successfully treated with intravenous BC-3781.[4–6] BC-3781 acts, like other pleuromutilin derivatives, by binding to the peptidyl transferase centre of the 50S subunit of ribosomes and cross-resistance to other antimicrobial classes is uncommon.[3]

In this study we describe the antibacterial *in vitro* activity of BC-3781 against a set of the most prevalent respiratory pathogens, including the atypical respiratory pathogens *L. pneumophila*, *M. pneumoniae* and *C. pneumoniae*.

Materials and 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 CARTIs from medical centres located in the USA and various European countries. Additionally, the following atypical respiratory pathogens were included: 30 clinical *L. pneumophila* isolates of five different serogroups collected in 2007–08 in Germany, 4 *M. pneumoniae* strains from the ATCC (ATCC 15531, ATCC 15293, ATCC 49894 and ATCC 29342), 50 clinical *M. pneumoniae* isolates collected in 2009–10 in Germany, 2 *C. pneumoniae* strains from the ATCC (ATCC VR-1310 and ATCC VR-1360) and 50 clinical *C. pneumoniae* isolates collected in 2009–10 in Germany.

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 CLSI recommendations (M07-A8, 2009).[10] Ninety-six-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*). CLSI and EUCAST interpretive criteria were used to categorize the isolates as susceptible, intermediate and resistant to comparator agents.[11,12] Concurrent testing of quality control 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) as described by Edelstein,[13] using an inoculum of 3×10^5 cfu/mL. Additionally, MIC values were determined by the agar dilution technique using BYEα containing 25% charcoal (BCYEα) and an inoculum size of 10^4–10^5 cfu/spot. MIC values were read after 3 days of incubation at 35°C in a humidified atmosphere.[14] *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 served as controls.

Susceptibility of *M. pneumoniae* was determined by broth microdilution in glucose-containing 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 0.5×10^4–10^5 colour changing units/mL as described previously.[15] The MIC was defined as the lowest concentration of antimicrobial that inhibited visible growth or metabolism of mycoplasma isolates (no colour change of medium) at the time when the antibiotic-free growth control showed a colour change.

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 inclusion forming units/mL) were treated with the test compounds dissolved in Iscove's modified Dulbecco's medium (Life Technologies, Grand Island, NY, USA) supplemented with L-glutamine, phenol red, HEPES, sodium hydrogen carbonate, fetal calf serum, MEM vitamin solution 100× (Life Technologies), non-essential amino acids, glucose and cycloheximide at 35°C in a 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, Bio-Rad, Austria). The MIC was defined as the lowest concentration of antibiotic at which no inclusions were observed.[16]

Results

BC-3781 was the most potent agent tested against this *S. pneumoniae* collection ($MIC_{50/90}$ 0.12/0.25 mg/L; Tables 1 and 2) when compared with other antimicrobial agents used

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

	Cumulative percentage of strains inhibited at each MIC (mg/L)							
Organism (no. tested)	0.015	0.03	0.06	0.12	0.25	0.5	1	2
S. pneumoniae (157)	3.2	7.6	34.4	80.9	99.4	100.0	—	—
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
β-lactamase negative (51)	—	—	—	—	7.8	56.9	92.2	100.0
β-lactamase positive (51)	—	—	—	—	7.8	56.9	82.4	100.0
M. catarrhalis (50)	2.0	4.0	74.0	100.0	—	—	—	—

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

Antimicrobial agent (no. tested)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	Percentage susceptible/resistant[a]
S. pneumoniae (157)				
BC-3781	0.12	0.25	0.015–0.5	-/-
penicillin[b]	0.25	>2	≤0.03 to >2	87.3/0.0
penicillin[c]	0.25	>2	≤0.03 to >2	34.4/33.1
erythromycin	0.5	>16	0.015 to >16	49.7/49.7
clindamycin	0.06	>16	≤0.008 to >16	68.2/31.2
azithromycin	2	>16	0.015 to >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 to >8	43.9/43.3
penicillin susceptible (MIC ≤0.06 mg/L; 54)				
BC-3781	0.12	0.25	0.015–0.25	-/-
erythromycin	0.06	4	0.015 to >16	83.3/14.8
clindamycin	0.06	0.06	≤0.008 to >16	94.4/5.6
azithromycin	0.12	16	0.015 to >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 to >8	83.3/7.4
penicillin intermediate (MIC 0.12–1 mg/L; 51)				
BC-3781	0.06	0.12	0.015–0.25	-/-
erythromycin	4	>16	0.015 to >16	39.2/60.8
clindamycin	0.06	>16	0.015 to >16	60.8/37.3
azithromycin	4	>16	0.03 to >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 mg/L; 52)				
BC-3781	0.12	0.25	0.015–0.5	-/-
erythromycin	>16	>16	0.03 to >16	25.0/75.0
clindamycin	>16	>16	0.03 to >16	48.1/51.9
azithromycin	>16	>16	0.06 to >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 to >8	7.7/90.4
H. influenzae (102)				
BC-3781	0.5	2	0.25–2	-/-
ampicillin	0.5	>4	≤0.25 to >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 to >8	81.4/15.7

Continued

Table 2. *Continued*

Antimicrobial agent (no. tested)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	Percentage susceptible/resistant[a]
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 to >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 to >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 to >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 (2011).[11]
[b]Criteria as published by the CLSI (2011) for 'Penicillin parenteral (non-meningitis)'.[11]
[c]Criteria as published by the CLSI (2011) for 'Penicillin (oral penicillin V)'.[11]
[d]Criteria as published by EUCAST (2011).[12]
[e]Included 40 β-lactamase-positive and 10 β-lactamase-negative isolates.
[f]Based on β-lactamase production.

to treat CABP, such as penicillin ($MIC_{50/90}$ 0.25/>2 mg/L), macrolides (erythromycin $MIC_{50/90}$ 0.5/>16 mg/L and azithromycin $MIC_{50/90}$ 2/>16 mg/L), doxycycline ($MIC_{50/90}$ 0.12/8 mg/L) and levofloxacin ($MIC_{50/90}$ 1/1 mg/L; Table 2). BC-3781 inhibited all *S. pneumoniae* isolates at ≤0.5 mg/L and was comparably potent against penicillin-susceptible ($MIC_{50/90}$ 0.12/0.25 mg/L), -intermediate ($MIC_{50/90}$ 0.06/0.12 mg/L) and -resistant ($MIC_{50/90}$ 0.12/0.25 mg/L) isolates (Table 1). In contrast, resistance to macrolides, clindamycin and trimethoprim/sulfamethoxazole among *S. pneumoniae* increased considerably as the penicillin MIC values increased. Among the penicillin-resistant (MIC ≥2 mg/L) isolates, macrolide resistance was 75.0% and >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 (Table 2).

BC-3781 also showed potent activity against the fastidious Gram-negative respiratory pathogens *H. influenzae* and *M. catarrhalis* (Tables 1 and 2). *H. influenzae* isolates were inhibited by BC-3781 at concentrations ranging from 0.25 to 2 mg/L. BC-3781 was similarly active against β-lactamase-negative ($MIC_{50/90}$ 0.5/1 mg/L) and β-lactamase-positive ($MIC_{50/90}$ 0.5/2 mg/L) *H. influenzae* isolates, with MIC distributions that were nearly identical (Table 1). Furthermore, BC-3781 was very active against *M. catarrhalis* regardless of β-lactamase production, with $MIC_{50/90}$ values of 0.06/0.12 mg/L and all isolates being inhibited at BC-3781 concentrations of ≤0.12 mg/L (Table 2). When compared with the other tested antimicrobials, BC-3781 was again

among the most active compounds, with activities comparable to those of β-lactam antibiotics or doxycycline (Table 2).

BC-3781 demonstrated good activity against *L. pneumophila*, with $MIC_{50/90}$ of 0.06/0.5 mg/L in BYEα (broth microdilution) and $MIC_{50/90}$ of 0.12/0.5 mg/L in BCYEα (agar dilution; Table 3). The activity of BC-3781 in BYEα was similar to that of erythromycin ($MIC_{50/90}$ 0.03/0.12 mg/L), but significantly less than that of azithromycin, moxifloxacin and levofloxacin ($MIC_{50/90}$ 0.015/0.015 mg/L for all three agents; Table 3). Charcoal supplementation of the test medium did not adversely affect the activity of BC-3781 ($MIC_{50/90}$ 0.12/0.5 mg/L), whereas a 4- to 8-fold increase was determined for the tested fluoroquinolones and azithromycin ($MIC_{50/90}$ of 0.06/0.12 mg/L for all three antibiotics), as reported previously.[14]

BC-3781 activity against *C. pneumoniae* was evaluated initially against two ATCC strains. For these two strains the MIC values were 0.01 and 0.04 mg/L, respectively, which indicated up to 16-fold greater activity than the tested macrolides and doxycycline and 128-fold greater activity than moxifloxacin (Table 3). Following this initial evaluation, BC-3781 was tested against 50 contemporary clinical isolates collected in Germany, which displayed MIC values ranging from 0.02 to 0.08 mg/L and $MIC_{50/90}$ values of 0.02/0.04 mg/L.

BC-3781 also exhibited potent activity against *M. pneumoniae*, with $MIC_{50/90}$ values of 0.006/0.006 mg/L when tested against 50 contemporary clinical isolates from Germany. The BC-3781 MIC range for this set of organisms was slightly higher than that demonstrated by the four ATCC strains evaluated separately (≤0.0003–0.0006 mg/L). When compared with reference antimicrobial agents for the treatment of respiratory *Mycoplasma* infections, BC-3781 was significantly more active than doxycycline, ciprofloxacin or clindamycin, and comparable to erythromycin and azithromycin (Table 3).

Discussion

CARTIs, especially CABP, are a significant cause of morbidity and mortality in all age groups. Although CABP may be caused by a wide variety of pathogens, a limited number of agents are responsible for most cases.[1] *S. pneumoniae* is the most frequently isolated pathogen, and other bacterial causes include *H. influenzae*, *M. catarrhalis* and *S. aureus*. The 'atypical' organisms are also recognized as important causes of CABP and include *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*.[1,2] Until more accurate and rapid diagnostic methods are available, the initial treatment for most patients with CABP and other CARTIs will remain empirical.[1]

In the present study, the novel pleuromutilin agent BC-3781 demonstrated excellent activity against the organisms most frequently responsible for CARTIs. BC-3781 inhibited all *S. pneumoniae* at ≤0.5 mg/L, with no difference noted between

Table 3. Antimicrobial activity of BC-3781 against the atypical respiratory pathogens *L. pneumophila*, *M. pneumoniae* and *C. pneumoniae*

Antimicrobial agent (no. tested)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)
L. pneumophila (30)[a]			
BC-3781	0.06 (0.12)[a]	0.5 (0.5)[a]	0.06–1 (0.12–1)
azithromycin	0.015 (0.06)[a]	0.015 (0.12)[a]	0.004–0.03 (0.06–0.5)
erythromycin	0.03 (0.06)[a]	0.12 (0.25)[a]	0.03–1 (0.06–1)
moxifloxacin	0.015 (0.06)[a]	0.015 (0.12)[a]	0.0018–0.03 (0.06–0.25)
levofloxacin	0.015 (0.06)[a]	0.015 (0.12)[a]	0.007–0.03 (0.06–0.25)
C. pneumoniae			
BC-3781 (50)	0.02	0.04	0.02–0.08
BC-3781 (2)	NA	NA	0.01–0.04
azithromycin (2)	NA	NA	0.08–0.16
clarithromycin (2)	NA	NA	0.02–0.08
erythromycin (2)	NA	NA	0.04–0.16
moxifloxacin (2)	NA	NA	0.32–1.28
doxycycline (2)	NA	NA	0.04–0.08
M. pneumoniae			
BC-3781 (50)	0.006	0.006	≤0.003–0.024
BC-3781 (4)	NA	NA	≤0.0003–0.0006
azithromycin (4)	NA	NA	0.00015–0.0003
erythromycin (4)	NA	NA	0.0025–0.005
clindamycin (4)	NA	NA	0.4–0.8
doxycycline (4)	NA	NA	0.04–0.04
ciprofloxacin (4)	NA	NA	0.2–0.8

NA, not applicable (due to the small number of strains tested).
[a]MICs determined by broth microdilution using BYEα; MICs in brackets show MICs determined by agar dilution using BCYEα.

penicillin-susceptible and -resistant subsets, and all *H. influenzae* isolates were inhibited by ≤2 mg/L, irrespective of β-lactamase production. *M. catarrhalis* were also very susceptible to BC-3781 (MICs ≤0.12 mg/L).

Furthermore, the BC-3781 antibacterial profile included atypicals such as *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*, activity being equal to or significantly greater than that of antibiotics currently in use.[13,14] The excellent activity of BC-3781 against *C. pneumoniae* indicates good intracellular activity and the favourable penetration properties needed for targeting intracellular pathogens in general. In summary, BC-3781 was very active against organisms commonly associated with CARTIs and was not negatively influenced by resistance to other antimicrobials. Further studies appear warranted to define the role of this novel pleuromutilin antibiotic in the treatment of CARTIs.

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