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Activity of BC-3781, a Novel Pleuromutilin Compound, Tested against Clinical Isolates of MRSA, Including Molecularly Characterized Community-Acquired and Hospital-Associated Strains

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ABSTRACT

Background: BC-3781 has a unique mode of action which interferes with bacterial protein synthesis without cross-resistance (R) to other antimicrobial classes. Pleuromutilins inhibit protein synthesis by binding the peptidyl transferase component of the 50S subunit of ribosomes. We tested the activity of BC-3781 against a diverse collection of *S. aureus.*

Methods: Antimicrobial activity of BC-3781 and comparators was determined against 924 non-duplicate *S. aureus*: 671 resistant (MRSA) and 253 susceptible (MSSA) to oxacillin. The MRSA collection includes strains from 2010 (103), 2009 (211), 2008 (110) and 2006 (102) as well as molecularly characterized strains from various years and geographic regions. The strains were cultured predominately from USA and European hospitals and tested for susceptibility against BC-3781 and comparators by the CLSI broth microdilution method (M07-A8).

Results: BC-3781 exhibited potent activity against MRSA with a MIC mode of 0.12 µg/mL for all subsets tested. Most MIC values (>98%) were in the range of 0.06 to 0.25 µg/mL. BC-3781 activity remained constant during the study period and was not affected by SCC*mec* type. USA300 (45) and USA400 (5) MRSA isolates were very susceptible to BC-3781. BC-3781 activity against MRSA was similar to that exhibited for MSSA.

Conclusions: BC-3781 demonstrated potent *in vitro* activity against a large and diverse collection of MRSA. BC-3781 displayed a narrow MIC range and its activity was not adversely influenced by R to other antimicrobial classes. These results indicate that BC-3781 should be further evaluated for treatment of MRSA infections.

Year of culture / molecular results (no. tested)	No. of isolates (%) with BC-3781 MIC [µg/mL]				
	0.03	0.06	0.12	0.25	0.5
MRSA					
2010 (103)		30 (29.1)	44 (42.7)	26 (25.2)	3 (2.9)
2009 (211)	7 (3.3)	80 (37.9)	97 (46.0)	25 (11.8)	2 (1.0)
2008 (110)		13 (11.8)	63 (57.3)	33 (30.0)	1 (0.9)
2006 (102)		12 (11.8)	65 (63.7)	23 (22.5)	2 (2.0)
USA300/400 (50)		3 (6.0)	47 (94.0)		
SCC*mec* type II (10)			8 (80.0)	2 (20.0)	
SCC*mec* type III (10)			9 (90.0)	1 (10.0)	
SCC*mec* type IV (75)		3 (4.0)	68 (90.7)	4 (5.3)	
MSSA (253)	2 (0.7)	36 (14.2)	201 (79.4)	13 (5.1)	1 (0.4)
All Results (924)	9 (1.0)	177 (19.2)	602 (65.1)	127 (13.7)	9 (1.0)

INTRODUCTION

It is well known that methicillin-resistant *Staphylococcus aureus* (MRSA) can also be refractory to many antimicrobial agents that are currently used in clinical practice. This compromises difficult empiric treatment decisions for infections caused by this commonly isolated pathogen. MRSA infections that do not respond well to the prescribed antimicrobial agents, which is not uncommon, can quickly become serious and may lead to hospitalization.

Community-associated MRSA (CA-MRSA) and healthcare-associated (HA-MRSA) can have distinct susceptibility patterns. However, it is becoming more difficult to distinguish between CA-MRSA and HA-MRSA due to the evolution of resistant clones that can be isolated within both of these environmental settings. HA-MRSA is considered to be a serious pathogen among very ill patients that is associated with numerous types of infections, and can be difficult to treat. CA-MRSA commonly causes wound infections, and it is recognized as the most problematic pathogen causing complicated skin and skin structure infections (cSSSI). Resistance to currently available antimicrobial classes continues to increase, and safe and effective novel treatment options are urgently needed for clinical use against this important bacterial pathogen.

BC-3781 is a pleuromutilin antimicrobial agent intended for systemic use that is currently in clinical phase II and is being targeted for treating cSSSI as well as bacterial pneumonia caused by MRSA and other drug-resistant bacteria. BC-3781, like other pleuromutilins, interferes with bacterial protein synthesis by binding the peptidyl transferase center of the 50S subunit of ribosomes. Cross resistance to other antimicrobial classes is unlikely due to unique interaction with the central part of domain V of 23S rRNA. One advantage of BC-3781, which is unique to other agents in its class, is that it can be dosed both orally and intravenously. Data that have been derived from a number of 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. This study documents the *in vitro* activity of BC-3781 against a large, geographically diverse collection of MRSA and MSSA, including CA- and HA-MRSA.

MATERIALS & METHODS

Bacterial isolates: A total of 924 non-duplicate isolates of *S. aureus* isolates were collected from medical centers located in the United States (USA) and Europe which were submitted to the SENTRY Antimicrobial Surveillance Program. Isolates were from documented patient infections with species identification confirmed by standard biochemical tests including BactiStaph® latex and tube coagulase agglutination tests (Remel, Lenxa, KS, USA), when needed.

Antimicrobial susceptibility testing: All isolates were tested for antimicrobial susceptibility using reference frozen-form panels with cation-adjusted Mueller-Hinton broth produced by JMI Laboratories (North Liberty, Iowa, USA). Tests were performed according to the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) using Mueller-Hinton broth (Becton Dickinson, Franklin Lakes, New Jersey, USA).

In addition to BC-3781, five comparator agents were tested against a subset of 712 recent clinical isolates (Table 2) and four additional agents were tested against the 50 isolates of confirmed USA clones of CA-MRSA (Table 3). Interpretive criteria for MIC values of comparison agents were those of the CLSI (M100-S20; 2010) or EUCAST (2009). Concurrent quality control (QC) testing of *S aureus* ATCC 29213 was performed per M07-A8 [2009]. MIC QC for BC-3781 was guided by earlier studies by JMI Laboratories and with the MIC ranges recently approved by the CLSI QC working group (June 2010) of the Antimicrobial Susceptibility Testing Subcommittee.

Molecular characterization: SCC*mec* types (I through VI) were determined using a multiplex PCR approach according to the protocol described by Milheirico et al. (2007). *S. aureus* carrying SCC*mec* types I–VI and *S. aureus* ATCC 29213 were concurrently tested for quality control purposes. Bacterial chromosomal DNA was digested with SmaI and subjected to pulsed-field gel electrophoresis (PFGE) as previously described by McDougal et al. (2003). Gel pattern analysis was performed using the GelCompar II software (Applied Math, Kortrijk, Belgium) and the patterns obtained compared to those of the major USA and international MRSA clones.

RESULTS

- Among all tested *S. aureus* isolates, the overall MIC_{50} and MIC_{90} values for BC-3781 were 0.12 and 0.25 µg/mL, respectively (Table 1).
- The MIC_{90} value for MSSA was two-fold lower (0.12 µg/mL) compared to that of the MRSA (0.25 µg/mL) isolates tested in this collection (Tables 1 and 2).
- Comparative data from additional antimicrobial agents demonstrated that BC-3781 was unaffected by resistance to azithromycin (60.4-61.4%) or clindamycin (21.9% among the subset collection of S. aureus (64.9% MRSA, Table 2).
- BC-3781 was four-fold more active against MRSA than vancomycin (MIC_{90}, 1 µg/mL) and eight-fold more active than linezolid (MIC_{90}, 2 µg/mL) as shown in Table 2.
- All strains were susceptible to these two comparison agents. Excellent antimicrobial activity was observed for BC-3781 (MIC range 0.06-0.12 µg/mL) against USA300 and USA400 strains of CA-MRSA (Table 3). These strains were generally resistant to macrolides and susceptible to other commonly used agents (10% resistance to levofloxacin).
- BC-3781 was eight-fold more active than vancomycin and 16-fold more active than linezolid against the CA-MRSA isolates (Table 3).

Table 1. Cumulative percentage of all *S. aureus* isolates inhibited at each tested MIC grouped by oxacillin susceptibility[a]

Organism type	Cumulative MIC % for BC-3781 [µg/mL]				
(no. tested)	0.03	0.06	0.12	0.25	0.5
MRSA (671)	1.0	22.1	81.8	98.8	100.0
MSSA (253)	0.7	15.0	94.5	99.6	100.0
All Results (924)	1.0	20.1	85.3	99.0	100.0

[a.] For detailed MIC information by year and resistance phenotype, please see the table in the abstract.

Table 2. Antimicrobial activity of BC-3781 compared to those of comparator antimicrobial agents against a subset of 712 *S. aureus* strains

Antimicrobial agent (no. tested)	MIC_{50} [µg/mL]	MIC_{90} [µg/mL]	Range [µg/mL]	CLSI[a] %S / %R	EUCAST[a] %S / %R
All strains (712)					
BC-3781	0.12	0.25	0.03 – 0.5	- / -	- / -
Azithromycin	16	>16	0.25 – >16	38.6 / 60.1	37.6 / 61.4
Clindamycin	0.12	>16	0.03 – >16	78.1 / 21.9	77.7 / 21.9
Linezolid	2	2	0.5 – 4	100.0 / -	100.0 / 0.0
Oxacillin	>2	>2	≤0.25 – >2	35.1 / 64.9	35.1 / 64.9
Vancomycin	1	1	0.25 – 2	100.0 / 0.0	100.0 / 0.0
MSSA (253)					
BC-3781	0.12	0.12	0.03 – 0.5	- / -	- / -
Azithromycin	1	16	0.25 – >16	83.8 / 15.8	81.8 / 16.2
Clindamycin	0.12	0.12	0.06 – >16	96.8 / 3.2	95.7 / 3.2
Linezolid	2	2	0.5 – 4	100.0 / -	100.0 / 0.0
Vancomycin	1	1	0.5 – 2	100.0 / 0.0	100.0 / 0.0
MRSA (459)					
BC-3781	0.12	0.25	0.03 – 0.5	- / -	- / -
Azithromycin	>16	>16	0.25 – >16	14.1 / 84.0	13.7 / 85.9
Clindamycin	0.12	>16	0.03 – >16	67.9 / 32.1	67.9 / 32.1
Linezolid	2	2	0.5 – 4	100.0 / -	100.0 / 0.0
Vancomycin	1	1	0.25 – 2	100.0 / 0.0	100.0 / 0.0

[a.] Criteria as published by the CLSI [2010] and EUCAST [2010].

Table 3. *In vitro* activity of BC-3781 in comparison to selected antimicrobial agents tested against 50 isolates of community-acquired MRSA[a]

Antimicrobial agent	MIC_{50} [µg/mL]	MIC_{90} [µg/mL]	Range [µg/mL]	% susceptible/ resistant[b]
BC-3781	0.12	0.12	0.06 – 0.12	- / -
Erythromycin	>16	>16	>16	0.0 / 100.0
Azithromycin	>16	>16	4 – >16	0.0 / 96.0
Clindamycin	0.12	0.12	0.06 – 0.12	100.0 / 0.0
Doxycycline	0.12	2	0.06 – 4	100.0 / 0.0
Levofloxacin	0.25	0.5	0.12 – 4	90.0 / 10.0
Vancomycin	1	1	0.5 – 2	100.0 / 0.0
Linezolid	2	2	1 – 2	100.0 / -
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	100.0 / 0.0

[a.] Included USA300 clone (45 strains) and USA 400 clone (five strains).
[b.] Criteria as published by the CLSI [2010]. - No available breakpoint criteria.

CONCLUSIONS

- BC-3781 was very active against *S. aureus*, including HA-MRSA and CA-MRSA. The vast majority (>85%) of the isolates tested in this investigation were inhibited at ≤0.12 µg/mL or less and 99.0% were inhibited by ≤0.25 µg/mL, with the highest BC-3781 MIC at 0.5 µg/mL.
- The overall potency of BC-3781 against *S. aureus* was four- to 16-fold greater than the next most potent agents tested (linezolid and vancomycin).
- BC-3781 shows promising activity against *S. aureus*, the most prevalent Gram-positive pathogen producing SSSI. Pending clinical phase II data, BC-3781 remains a promising adjunct for management of acute bacterial SSSI.

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