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In Vitro Synergy/Antagonism of the Pleuromutilin BC-3781 with Selected Antibiotics Against Gram-Positive and Gram-Negative Bacteria

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ABSTRACT

Background: The novel semisynthetic pleuromutilin antibiotic BC-3781 is in development for the treatment of skin and respiratory infections. BC-3781 exhibits potent activity against Gram-positive and fastidious Gram-negative organisms predominantly causing these infections but is inactive against *Enterobacteriaceae* and non-fermenters. In this study, potential synergy/antagonism of BC-3781 with various antibiotics was evaluated by determining fractional inhibitory concentrations (FIC).

Methods: Susceptibility to BC-3781 was evaluated in combination with the following antibiotics by broth microdilution (CLSI M7): vancomycin, linezolid, levofloxacin, gentamicin, ceftriaxone, tigecycline, doxycycline, azithromycin, trimethoprim/sulfamethoxazole, clindamycin, chloramphenicol, quinupristin/dalfopristin, daptomycin, aztreonam, piperacillin/ tazobactam, meropenem and amikacin for *S. aureus* (*n* = 6); penicillin, ceftriaxone, levofloxacin, erythromycin, ampicillin, vancomycin, meropenem, aztreonam, piperacillin/ tazobactam and amikacin for *S. pneumoniae* (*n* = 6), *S. pyogenes* (*n* = 3) and *S. agalactiae* (*n* = 3); amoxicillin/clavulanic acid, ceftriaxone, trimethoprim/sulfamethoxazole, azithromycin and chloramphenicol for *H. influenzae* (*n* = 6); aztreonam, piperacillin/ tazobactam, meropenem and amikacin for *Enterobacteriaceae* (*n* = 10) and *P. aeruginosa* (*n* = 2).

Results: BC-3781 exhibited no antagonistic effect with any antibiotic tested against any bacterial strain tested. The effect was largely indifferent/additive with FIC indices (FICI) of 0.5-4 and mean FICI typically being close to 1. No apparent synergy was observed with the exception of trends towards synergy observed across *S. aureus* isolates when BC-3781 was combined with doxycycline (in 5 of 6 isolates) and across *S. pneumoniae* (6 of 6 isolates) when BC-3781 was combined with aztreonam.

Conclusions: Overall, BC-3781 was confirmed to have largely 'no interaction', neither antagonism nor synergy, when combined with other antibiotics against Gram-positive or Gram-negative organisms, including those with important resistance phenotypes (e.g. MRSA and ESBL) suggesting that there is no potential issue for combination therapy when Gram-negative coverage is necessary.

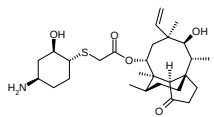
INTRODUCTION

BC-3781 is a novel pleuromutilin antibiotic with an antibacterial profile covering the most prominent bacterial pathogens causing acute bacterial skin and skin structure infections (ABSSSI) and community acquired bacterial pneumonia (CABP).[1] It comprises activity against Gram-positive cocci such as *Staphylococcus* spp. and *Streptococcus* spp. including multi-drug resistant isolates and also fastidious Gram-negative organisms such as *Haemophilus* spp. and *Moraxella catarrhalis*, as well as against atypical organisms like *Legionella pneumophila, Mycoplasma pneumoniae and Chlamydophila pneumoniae*. To date more than 400 people were exposed to BC-3781 administered intravenously or orally in sixteen clinical Phase 1 studies and one Phase 2 study.

Phase 2 study in patients with complicated skin and skin structure infections demonstrated that the activity of BC−3781 was comparable to that of vancomycin.[2,3] BC-3781 is well tolerated and shows a good safety profile irrespective of the administration route.

ABSSSI and CABP are primarily caused by the above mentioned bacterial pathogens. However, Gram-negative species such as *Enterobacteriaceae* or *Pseudomonas aeruginosa* might play a role in these infections as well. Thus combination therapy with agents covering these organisms might be necessary. Additionally, patients might have been treated with other antibacterial agents before being exposed to new therapy. This study therefore evaluated the potential of synergy/antagonism of BC-3781 with a number of relevant antibacterials against a set of Gram-positive and Gram-negative species.

Figure 1. Structure of BC-3781.



METHODS

BC-3781 was supplied by Nabriva Therapeutics AG. All other test compounds as listed in Table 1 were purchased from commercial sources.

Synergy/antagonism was determined by Checkerboard broth microdilution technique according to CLSI (M07-A9, 2012) using the following growth media: CAMHB for *S. aureus*, CAMHB supplemented with 3% lysed horse blood for *Streptococcus* spp. and HTM for *H. influenzae*. For testing of daptomycin, the Ca^{2+} concentration of CAMHB was adjusted to 50 mg/L. The final inoculum of $5x10^5$ CFU/ml was prepared by the colony suspension method according to CLSI.

Concurrent MIC testing of QC organisms was performed using *S. aureus* ATCC29213, *S. pneumoniae* ATCC 49619, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *H. influenzae* ATCC49427 and using BC-3781 QC limits as approved by the CLSI and published earlier.[4]

Fractional inhibitory concentrations indices (FICI) were calculated using the following equation: $FICI=FIC_{compoundA}/MIC_{compoundA} + FIC_{compoundB}/MIC_{compoundB}$. Using the criteria described by Odds, the FICI and mean FICI for the combination were interpreted as follows: ≤0.5 = synergy, >0.5-4 = additive/indifferent and >4 = antagonism. [5]

RESULTS

S. aureus

- BC-3781 displayed potent activity against *S. aureus* (incl. MRSA) with MICs of 0.12 mg/L against all strains tested including fluoroquinolone- and macrolide-resistant MRSA.
- Mean FICI were typically 1-2 indicating an indifferent/additive effect for BC-3781 with the tested antibiotics. FICIs for BC-3781 in combination with doxycline (5 of 6 strains had FICIs ≤0.5; Table 1) as well as the resulting isobologram (Figure 2) suggest a slight trend towards synergistic interaction.
- No antagonism was observed for any antibiotic tested in combination with BC-3781.

Table 1. MIC [µg/ml] and FICI for BC-3781 in combination with the indicated drug

Compound / Organism	MIC Range [µg/ml]	Mean FICI range in combination with BC-3781	Synergy/antagonism
***S. aureus* [50% MRSA] (*n*=6)**			
BC-3781[1]	0.12	-	-
Amikacin	2-64	0.64-2.63	IND/ANT for 1 strain
Azithromycin	1->128	0.96-1.13	IND
Aztreonam	>256	1.13-1.29	IND
Ceftriaxone	4->128	0.71-1.29	IND
Chloramphenicol	8	1.13-1.63	IND
Clindamycin	0.12->8	0.88-1.29	IND
Daptomycin	2	1.29-1.96	IND
Doxycycline	0.12-2	0.55-0.83	IND/SYN for 5 strains
Gentamicin	0.5->32	0.79-2.29	IND
Levofloxacin	0.25-16	1.13-1.63	IND
Linezolid	2-4	0.86-1.29	IND
Meropenem	0.12-16	0.98-1.38	IND/SYN for 1 strain
Pip/tazo	0.5/4-64/4	0.67-1.14	IND
Quinupristin/Dalfopristin	0.25-0.5	0.96-1.29	IND
Sulfamethoxazole	0.06/1.14->16/304	1.00-2.29	IND
Tigecycline	0.12	0.56-0.88	IND/SYN for 1 strain
Vancomycin	0.5-1	1.13-1.96	IND
***S. pneumoniae* [33% PRSP; 50% PISP] (*n*=6)**			
BC-3781[2]	0.12-0.5	-	-
Amikacin	32-128	0.78-1.07	IND/SYN for 2 strains
Ampicillin	0.015->2	1.00-1.61	IND/SYN for 1 strain
Aztreonam	128->256	0.43-1.02	IND/SYN for 6 strains
Ceftriaxone	0.015-2	0.93-1.16	IND
Erythromycin	0.03->256	0.68-1.16	IND
Levofloxacin	0.5-2	0.91-1.16	IND
Meropenem	0.015-2	1.07-1.14	IND
Penicillin	0.015-4	0.76-1.08	IND/SYN for 1 strain
Piperacillin/Tazobactam	0.03/4-8/4	0.80-1.01	IND/SYN for 1 strain
Vancomycin	0.25-0.5	1.03-1.07	IND/SYN for 1 strain
ß-hemolytic *Streptococcus spp.* [50% *S. pyogenes*; 50% *S. agalactiae*] (*n*=6)			
BC-3781[3]	0.03-0.06	-	-
Ceftriaxone	0.015-0.12	0.88-1.63	IND
Erythromycin	0.03->256	0.86-1.40	IND
Levofloxacin	0.25-1	1.30-1.97	IND
Penicillin	0.008-0.12	0.94-1.84	IND
Vancomycin	0.25-0.5	1.13-1.30	IND
***H. influenzae* [33% ß-lactamase positive] (*n*=6)**			
BC-3781[4]	0.25-2	-	-
Amoxicillin/clavulanic acid	1/0.5-4/2	0.98-1.23	IND
Azithromycin	0.5-2	1.11-1.36	IND
Ceftriaxone	0.002-0.06	0.86-2.29	IND
Chloramphenicol	0.5	1.11-1.61	IND
Sulfamethoxazole	≤0.03/0.57-8/152	0.43-1.48	IND/SYN for 1 strain
***Enterobacteriaceae and P. aeruginosa* (*n*=12) [6]**			
BC-3781[5]	16->32	-	-
Amikacin	1-8	0.82-2.00	IND/SYN for 1 *M. morganii* strain
Aztreonam	0.06->128	0.28-1.99	IND/SYN for 1 *E.coli* strain
Meropenem	0.015-4	0.69-1.43	IND/SYN for 1 *K. pneumoniae* strain
Piperacillin/Tazobactam	1/4-256/4	0.64-2.43	IND/ANT for 1 *K. oxytoca* strain

IND, indifferent; SYN, synergy; ANT, antagonism

[1], MIC range presented from 17 test results for each organism evaluated
[2], MIC range presented from 10 test results for each organism evaluated
[3], MIC range presented from 5 test results for each organism evaluated
[4], MIC range presented from 5 test results for each organism evaluated
[5], MIC range presented from 4 test results for each organism evaluated
[6], The following organisms were included (number of isolates): *E. coli* (3), *K. pneumoniae* (2), *K. oxytoca* (1), *P. mirabilis* (1), *E. cloacae* (1), *E. aerogenes* (1), *M. morganii* (1), *P. aeruginosa* (2)

RESULTS continued

S. pneumoniae

- BC-3781 showed good activity against *S. pneumoniae* with all isolates inhibited at BC-3781 MICs of 0.12-0.5 mg/L irrespective of resistance to penicillin/ß-lactams or macrolides (Table 1).
- Mean FICIs were close to 1 indicating an indifferent/additive effect for BC-3781 and the tested antibiotics for all strains except one with a mean FICI of 0.43 indicative for synergy. Individual FICI values <0.5 were observed for single strain/drug combinations, whereas a trend towards synergy was noted with aztreonam with all six evaluated isolates having at least one FICI <0.5 (Figure 3).
- As for *S. aureus* no antagonism was observed for any antibiotic tested in combination with BC-3781.

Figure 2. Isobologram for BC-3781 (drug B) in combination with doxycycline (drug A) against *S. aureus*

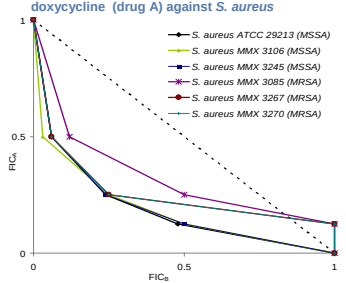
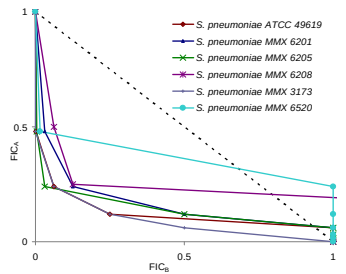


Figure 3. Isobologram for BC-3781 (drug B) in combination with aztreonam (drug A) against *S. pneumoniae*



H. influenzae

- BC-3781 displayed potent activity against *H. influenzae* including ß-lactamase producing isolates. MICs ranged from 0.25-2 mg/L (Table 1).
- Mean FICIs and isobolograms indicated an indifferent/additive effect for all isolates/drug combinations except one isolate where a mean FICI of 0.43 was observed for the combination of BC-3781 and sulfamethoxazole.
- No antagonisms was observed for any antibiotic tested with BC-3781.

Enterobacteriaceae and *P. aeruginosa*

- MIC values of ≥16 mg/L confirmed the lack of activity of BC-3781 against *Enterobacteriaceae* and *P. aeruginosa* (Table 1).
- Excluding one ESBL producing *E. coli* where a mean FICI of 0.28 indicative of synergy of BC-3781 and aztreonam was observed mean FICIs were typically close to 1 indicating an indifferent/additive effect.
- No antagonism was seen with BC-3781 in combination with any of the agents tested.

CONCLUSIONS

- When BC-3781 was combined with other agents, the effect was largely indifferent/additive with the exception of a trend towards synergy observed across evaluated isolates of *S. aureus* when BC-3781 was combined with doxycycline and with *S. pneumoniae* when BC-3781 was combined with aztreonam. Some exceptions were also observed for single strain/antibiotic combinations when single FIC values were close to or below 0.5
- BC-3781 was confirmed not to have any antagonistic effect on the activity of combination agents against Gram-positive or Gram-negative pathogens, including those with important resistance phenotypes (e.g. MRSA and ESBL).
- The results of this study suggest no potential issue for combination therapy with BC-3781 when Gram-negative coverage is necessary.

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