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In Vitro Activity of the Novel Pleuromutilin BC-3781 Tested Against Bacterial Pathogens Causing Sexually Transmitted Diseases (STD)

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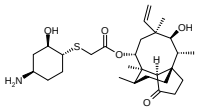
BC-3781



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AMENDED ABSTRACT

Background: STD are a significant health challenge in Europe and the USA. *Chlamydia trachomatis* infection and gonorrhea caused by *Neisseria gonorrhoeae* are the most frequently reported sexually transmitted and reportable diseases in both areas with high infection rates among young persons particularly women. Decreased susceptibility of *N. gonorrhoeae* isolates to azithromycin, cefixime and ceftriaxone, recommended therapies for gonorrhea, is extremely concerning. BC-3781 is a novel pleuromutilin for oral and intravenous administration as treatment of bacterial skin and respiratory infections. This study evaluated its *in vitro* activity against the most prevalent bacterial pathogens causing STD.

Methods: The antibacterial activity of BC-3781 and comparators was tested against *C. trachomatis* (serovars A-K, L2,L3 and SA2f; n=15) in McCoy and HeLa229 cells and *Mycoplasma genitalium* (n=6), *M. hominis* (n=1), *Ureaplasma urealyticum* (n=1) by broth microdilution methods. *N. gonorrhoeae* (n=24), the anaerobe *Peptostreptococcus* spp. (n=10) and *Prevotella* spp. (n=10) were tested by agar dilution methods (CLSI).

Results: BC-3781 demonstrated potent activity against *C. trachomatis* including serovars causing lymphogranuloma venerum with $MIC_{50/90}$ of 0.02/0.04 µg/mL (range 0.01−0.04 µg/mL). This was comparable to the activity of moxifloxacin, clarithromycin and doxycycline and 5-fold more active than azithromycin or erythromycin ($MIC_{50/90}$, 0.1/0.2 µg/mL). BC−3781 was also active against *N. gonorrhoeae* ($MIC_{50/90}$, 0.125/0.5 µg/mL) with ciprofloxacin- and tetracycline-resistant strains being inhibited by BC-3781. BC−3781 demonstrated also activity against *Mycoplasma* spp. including the macrolide- resistant *M. hominis* (0.04 µg/mL), multi-drug resistant *M. genitalium* (0.016-0.063 µg/mL) and against *U. urealyticum* (1.6 µg/mL). All *Peptostreptococcus* spp. and 80% of *Prevotella* spp. isolates were inhibited at BC−3781 concentrations of ≤2 µg/mL. *Porphyromonas* spp. was highly susceptible to BC-3781 with MICs of 0.03 µg/mL. Good BC-3781 activity was also demonstrated against *H. ducreyi* (MIC ≤0.015-0.25 µg/mL).

Conclusion: Overall, BC-3781 displayed potent activity against the most relevant bacterial pathogens causing STD warranting further investigations on the potential of BC−3781 in this indication.

INTRODUCTION

Sexually transmitted diseases (STD) are a significant health challenge in the USA and in Europe. Infections caused by the bacterium *Chlamydia trachomatis* accounted for 1.4 million cases in 2011 in USA. The US Centers for Disease Control and Prevention (CDC) reports gonorrhea (GC) (caused by *Neisseria gonorrhoeae*) as one of the most common sexually transmitted diseases in the US, with more than 800,000 cases estimated to occur each year.[1] Overall, chlamydia and GC are the most frequently reported sexually transmitted and reportable infections in both Europe and the USA with high occurrence rates among young persons, particularly women.[1,2] WHO estimated ~105 million *C. trachomatis* and ~106 million GC infections globally in 2008.[3] Left untreated, these infections can cause serious health problems, particularly for women, including chronic pelvic pain, life-threatening ectopic pregnancy, pelvic inflammatory disease and infertility.[3] Gonorrhea infection also increases a person's risk of contracting and transmitting HIV.[4]

Control strategy relies on effective antibiotic therapy. STD organisms are progressively developing resistance to the antibiotic drugs prescribed to treat them: sulfonilamides, penicillin, cephalosporins, azithromycin, tetracycline, and ciprofloxacin. GC resistant to all of these antibiotics have been reported globally.[5] New antibacterials overcoming those infections, preferably available as oral formulations, are therefore urgently needed.

BC−3781 is a novel pleuromutilin antibiotic in development for oral and intravenous administration in treatment of bacterial skin and respiratory infections. The presented study demonstrated the activity of BC−3781 against the most prevalent bacterial pathogens causing STD. This includes *C. trachomatis*, gonococci, mycoplasmas, ureaplasmas and anaerobic cocci and bacilli causing gonorrhea, non-gonococcal urethritis, cervicitis and pelvic inflammatory disease.

METHODS

C. trachomatis serovars L_2/434 (ATCC VR-902B), L_2/404 (ATCC VR-903), A/G-17, B/TW-5, Ba/AP-2 (ATCC VR-347), E/UW-5, G/UW-57 (ATCC VR-878), SA_2f were cultured in McCoy cells (ATCC CRL-1696) and the serovars C/TW-3 (ATCC VR-578), D/UW-3 (ATCC VR-885), F/MRC 301, J/UW-36 (ATCC VR-886), K/UW-31 (ATCC VR-887) were cultured in HeLa 229 cells (ATCC CCL-2.1). MIC determinations were performed essentially as described earlier[6] on monolayers of the respective eukaryotic cells infected with *C. trachomatis* [10^2-10^3 inclusion forming units (IFU) per $3x10^5$ cells] on glass cover slips at drug concentrations ranging from 12.8-0.0003 µg/mL. After incubation at 35°C (5% CO_2) for 49-72 h the cells were fixed in methanol and inclusions were stained with alcoholic iodine solution. The MIC was defined as the lowest amount of antibiotic at which no inclusion was observed.

Susceptibility testing of *M. genitalium* (ATCC 33530), *M. hominis* (ATCC 23114), *U. urealyticum* (ATCC 27814) was performed by broth microdilution as described earlier.[7-9] Initial MICs were read when the change of color in the broth was first observed in the control wells (typically after 5-7 days). Susceptibility testing of the multi-drug resistant *M. genitalium* isolates was performed using the Vero cell culture and quantitative real-time PCR method as described earlier.[10] Here, the MIC was expressed as the minimal concentration of the test-antibiotic causing a 99% inhibition of growth when compared to the mean of the control. *N. gonorrhoeae* testing was performed by agar dilution technique as described by CLSI (M07-09, 2012). *N. gonorrhoeae* ATCC 49226 was included as a QC reference strain with all results being within established limits. *Haemophilus ducreyi* MIC determination was performed as described earlier.[11,12] MIC determination against the anaerobic organisms was performed by agar dilution as described by CLSI (M11-A8, 2012).

RESULTS

C. trachomatis

- BC-3781 exhibited potent activity against the intracellular *C. trachomatis* including serovars A, B, Ba, C causing ocular trachoma, serovars D, E, F, G, J, K causing oculogenital infections and the LGV strains L_2, L_3 and SA_2f causing lymphogranuloma venerum with $MIC_{50/90}$ of 0.02/0.04 µg/mL (Table 1). This was comparable to doxycycline, moxifloxacin and clarithromycin and 5-fold more active than azithromycin or erythromycin.

N. gonorrhoeae

- Against *N. gonorrhoeae* BC-3781 displayed $MIC_{50/90}$ of 0.12/0.5 µg/mL. It was fully active against fluoroquinolone-, tetracycline- and aminoglycoside-resistant isolates (Table 1).

H. ducreyi

- BC-3781 demonstrated potent activity against *H. ducreyi* causing chancroid, a sexually transmitted infection common in Africa and SE Asia. It displayed a MIC range of ≤ 0.015-0.25 µg/mL, which was comparable to the activity of tetracyclines, erythromycin and ß-lactam antibiotics (Table 1).

Anaerobic organisms

- When tested against the anaerobic *Peptostreptococcus* spp., *Prevotella* spp., *Porphyromonas* spp., which are commonly involved in pelvic inflammatory disease among other female genital tract infections, soft tissue infections, abscesses and infections of the oral cavity, BC-3781 displayed potent activity with all *Peptostreptococcus* spp. and *Prevotella* spp. being inhibited at BC-3781 concentration of ≤ 2 µg/mL and all *Porphyromonas* spp. strains at 0.03 µg/mL (Table 1).

Mycoplasma genitalium and other *Mycoplasma*/*Ureaplasma* spp.

- BC-3781 showed good activity against the tested *Mycoplasma* species with all isolates being inhibited at a BC-3781 concentration of ≤ 0.06 µg/mL. When compared with standard of care medications, BC-3781 was among the most active compounds (Table 2).
- When tested against very recent multi-drug resistant clinical isolates from patients failing treatment with high doses of azithromycin, moxifloxacin, and doxycycline, BC-3781 displayed potent activity with MICs ranging from 0.016-0.063 µg/mL.

Table 1. $MIC_{50/90}$ [µg/mL] of BC-3781 and comparator antibiotics against *C. trachomatis*, *N. gonorrhoeae*, *H. ducreyi* and anaerobic organisms

Species	*n*	MIC_{50}	MIC_{90}	Range	% S/R[e]
C. trachomatis, serovars A-I [a]					
BC-3781	15	0.02	0.04	0.01-0.04	-/-
Linezolid	15	12.8	>12.8	1.6->12.8	-/-
Azithromycin	13	0.1	0.2	0.025-0.2	-/-
Clarithromycin	13	0.005	0.01	0.0006-0.01	-/-
Doxycycline	13	0.01	0.02	≤0.005-0.02	-/-
Erythromycin	13	0.1	0.2	0.025-0.4	-/-
Moxifloxacin	13	0.05	0.05	≤0.025-0.05	-/-
N. gonorrhoeae					
BC-3781	24	0.12	0.5	0.03-1	-/-
Azithromycin	24	0.12	0.5	0.03-0.5	-/-
Cefixime	24	0.015	0.03	≤0.008-0.06	100.0/0
Ceftriaxone	24	≤0.008	0.03	≤0.008-0.03	100.0/0
Ciprofloxacin	24	0.008	**>4**	≤0.008-**>4**	87.5/12.5
Streptomycin	24	32	**>128**	8-**>128**	-/-
Tetracycline	24	0.5	**4**	0.12-**4**	41.7/29.2
H. ducreyi					
BC-3781	6	-	-	≤0.015-0.25	-/-
Amoxicillin/Clavulanic acid	6	-	-	0.06-1	-/-
Ceftriaxone	6	-	-	≤0.015	-/-
Ciprofloxacin	6	-	-	≤0.015	-/-
Doxycycline	6	-	-	0.12-16	-/-
Erythromycin	6	-	-	≤0.015-0.12	-/-
Tetracycline	6	-	-	≤0.015-32	-/-
Tigecycline	6	-	-	0.06-0.25	-/-
Peptostreptococcus spp.[b]					
BC-3781	10	0.06	1	0.03-2	-/-
Clindamycin	10	0.5	2	0.12-**>32**	90.0/10.0
Imipenem	10	0.06	0.12	≤0.03-0.5	100.0/0
Metronidazole	10	1	1	0.12-2	100.0/0
Prevotella spp.[c]					
BC-3781	10	0.5	4	0.015->16	-/-
Clindamycin	10	≤0.03	>32	≤0.03-**>32**	80.0/20.0
Imipenem	10	≤0.03	0.12	≤0.03-0.25	100.0/0
Metronidazole	10	2	4	0.5-4	100.0/0
Porphyromonas spp.[d]					
BC-3781	10	0.03	0.03	0.03	-/-
Clindamycin	10	≤0.03	0.06	≤0.03-0.06	100.0/0
Imipenem	10	≤0.03	0.06	≤0.03-0.06	100.0/0
Metronidazole	10	≤0.03	0.06	≤0.03-0.06	100.0/0

*, contains serovars A, B, Ba,C, D, E, F, G, J, K, L2, L3, SA2f (LGV lab strain), H, I; MIC test in HeLa229 and McCoy cells

[b], includes: *Finegoldia magna* (3), *P. anaerobius* (1), *P. asaccharolyticus* (2), *P. micros* (2), and *P. tetradius* (2)

[c], includes: *P. bivia* (4), *P. intermedia* (2), *P. melaninogenica* (2) and *P. oralis* (2)

[d], includes: *Porphyromonas gingivalis* (9), and unspeciated *Porphyromonas* (1)

[e], Criteria as published by the CLSI [2013]

Table 2. MIC [µg/mL] of BC-3781 and comparator antibiotics against *Mycoplasma spp.* and *Ureaplasma* spp.

Species	Strain	BC-3781	Azithro-mycin	Erythro-mycin	Clinda-mycin	Doxy-cycline	Moxi-floxacin	Cipro-floxacin	Reference
M. genitalium	M6489	0.063	>16	>16	ND	1	>16	>16	
M. genitalium	M6711	0.063	>16	≥64	ND	1	8	>16	
M. genitalium	M6712	0.063	>16	≥64	ND	1	8	>16	Jensen JS (unpublished data)
M. genitalium	M6714	0.016	>16	≥64	ND	1	4	16	
M. genitalium	M6735	0.063	>16	≥64	ND	1	8	16	
M. genitalium	ATCC 33530	0.001	0.001	0.01	0.4	0.08	ND	1.6	
M. hominis	ATCC 23114	0.04	6.4	>128	0.2	0.04	ND	1.6	Nabriva (unpublished data)
U. urealyticum	ATCC 27814	1.6	0.8	0.4	16	0.32	ND	2	

CONCLUSIONS

- The high potency of BC-3781 against *N. gonorrhoeae*, *C. trachomatis*, *Mycoplasma* species, *H. ducreyi* and anaerobic organisms suggest that BC-3781 could be a promising first-line antibiotic for the treatment of STD such as gonorrhea, non-gonococcal urethritis, cervicitis, chancroid and pelvic inflammatory disease, especially in populations with high resistance rates to standard of care antibiotics.
- Sexually transmitted diseases represent a major public health crisis; there is multi-drug resistance present and adequate oral therapies are lacking.
- As BC-3781 is available as an intravenous as well as oral formulation (tablet) and active against multi-drug resistant bacterial isolates, further studies are warranted to explore the BC-3781 activity against larger collections of isolates and to demonstrate its activity in human clinical STD trials.

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