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Antibacterial *In Vitro* Activity of Novel Extended Spectrum Pleuromutilins Against Gram-Positive and -Negative Bacterial Pathogens

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ABSTRACT

Objectives: The dramatic increase and rapid spread of multi-drug resistance (MDR), particularly carbapenemase and ESBL producing *Enterobacteriaceae*, MDR *Streptococcus pneumoniae* and MRSA raises the need for new treatment options to fight serious infections caused by these organisms and associated with high morbidity and mortality. This study investigated the antimicrobial activity of various novel derivatives of the new generation of pleuromutilin antibiotics - the extended spectrum pleuromutilins (ESP). The antibacterial profile of ESP expands on the conventional pleuromutilin spectrum covering additional major Gram-negative pathogens like *Escherichia coli*, *Klebsiella pneumoniae* and other *Enterobacteriaceae* along with staphylococci, streptococci, *Haemophilus influenzae* and atypical respiratory pathogens.

Methods: The antibacterial activity was determined for 14 novel semisynthetic ESP derivatives and comparator antibiotics by broth microdilution (CLSI, M7-A9). The bacterial spectrum included *E. coli* (*n* = 4), *K. pneumoniae* (*n* = 3), *Citrobacter freundii* (*n* = 2), *Enterobacter cloacae* (*n* = 2), *Staphylococcus aureus* (*n* = 1), *S. pneumoniae* (*n* = 2) and *H. influenzae* (*n* = 2). Additionally, $MIC_{50/90}$ were determined for eight compounds against larger sets of *E. coli* (*n* = 32) including ESBL (TEM, CTX-M) producing strains (78.1 %), *K. pneumoniae* (*n* = 24; 25.0 % ESBL producing), *S. pneumoniae* (*n* = 30; 76.7 % macrolide resistant) as well as against the NDM-1 *Enterobacteriaceae* panel (ATCC MP−18).

Results: All novel ESP derivatives tested demonstrated potent activity against the tested organisms. BC-9529, one of the most active derivatives, showed the following MIC ranges: *E. coli* (0.25-1 µg/mL), *K. pneumoniae* (0.5-2 µg/mL), *E. cloacae* (0.5-1 µg/mL), *S. aureus* (≤0.03 µg/mL), *S. pneumoniae* (0.125-8 µg/mL), *H. influenzae* (2-4 µg/mL). ESP derivatives were fully active against TEM-, CTX-M and NDM-1 producing *E. coli* (BC-9529, $MIC_{50/90}$ 0.5/1 µg/mL) which were largely resistant to amoxicillin/clavulanic acid ($MIC_{50/90}$ 16/>32 µg/mL), ceftriaxone ($MIC_{50/90}$ >16/>16 µg/mL), ceftazidime ($MIC_{50/90}$ 32/>32 µg/mL), ciprofloxacin ($MIC_{50/90}$ 16/>16 µg/mL) and doxycycline ($MIC_{50/90}$ 8/32 µg/mL). The activity of ESP was comparable to that of tigecycline ($MIC_{50/90}$, 0.25/0.5 µg/mL). BC-9529 showed also potent activity against *K. pneumoniae* ($MIC_{50/90}$, 1/2 µg/mL; tigecycline $MIC_{50/90}$, 2/4 µg/mL) including ß-lactamase producers and tetracycline-resistant isolates. Moreover, ESP derivatives were fully active against the NDM-1 metallo-ß-lactamase producing *Enterobacteriaceae*. All tested *S. pneumoniae* isolates were inhibited by ESP at concentrations ≤1 µg/mL irrespective of resistance to macrolides or penicillin.

Conclusions: ESP, the new generation of pleuromutilin antibiotics, demonstrated a potent antibacterial profile covering the most prevalent Gram-positive and Gram-negative bacterial organisms including multi-drug resistant and carbapenemase-producing strains. The additional coverage of *Enterobacteriaceae* represents a major extension of the antibacterial profile of conventional pleuromutilins which might lead to an additional treatment option for patients with infections caused by multi-drug resistant organisms such as carbapenemase producing *E. coli* and *K. pneumoniae*.

METHODS

The novel ESP are semi-synthetically derived from pleuromutilin, a homochiral natural fermentation product and made by medicinal chemistry at Nabriva Therapeutics.

MIC were determined by broth microdilution using CA-MHB according to CLSI M7-A9 (2012).

Bacterial strains were kindly provided by various sources: ESBL-producing *E. coli* and *S. pneumoniae* by F.J. Schmitz (Klinikum Minden, Germany); CTX-M ß-lactamase producing *E. coli* by D. Livermore (HPA, London); NDM-1 panel and KPC-2/-3 producing isolates by ATCC *S. aureus* and *S. pneumoniae* by JMI Laboratories;

INTRODUCTION

Extended spectrum pleuromutilins (ESP) are a novel generation of pleuromutilin antibiotics displaying a broad antibacterial profile including multi-drug resistant *Enterobacteriaceae* in addition to the profile of conventional pleuromutilins. Conventional pleuromutilin derivatives such as BC-3781 or retapamulin display potent activity against staphylococci, streptococci, *Haemophilus* spp., *Legionella pneumophila*, *Mycoplasma* spp., *Chlamydia* spp. and *Neisseria gonorrhoeae* among others but lack activity against *Enterobacteriaceae*.[1-4]

Table 1. Antibacterial activity of ESP against Gram-positive and -negative organisms

Compound / Species (*n*)	MIC range [µg/mL] *E. coli* (4)	*C. freundii* (2)	*K. pneumoniae* (3)	*E. cloacae* (2)	*S. aureus* (1)	*S. pneumoniae* (2)	*H. influenzae* (2)
Tigecycline	0.12-0.5	0.5-1	0.25-1	0.25-0.5	0.25	0.12	0.06-0.25
Linezolid	**>32**	**>32**	**>32**	**>32**	4	2	**8-16**
BC-7641	1-2	2-4	2	1-2	≤0.03	1-32	8-16
BC-9074	0.12-0.25	0.25-1	0.5-2	0.12-1	0.06	0.5->32	1-4
BC-9077	0.12	0.12-0.5	1-2	0.25-1	≤0.03	0.5->32	1-4
BC-9505	0.25-0.5	0.5-1	16->32	2-8	0.015	0.5-4	4-8
BC-9514	0.5-2	1-2	1-8	0.5-1	≤0.03	0.5-8	4-8
BC-9520	0.5-1	1	2-8	2	≤0.03	0.25-2	2
BC-9529	0.25-1	0.5-1	1-2	1	≤0.03	0.25-8	2-4
BC-9539	0.5-1	1	1	0.5-1	≤0.03	0.125-4	4-8
BC-9540	0.25-1	0.5-1	0.5-1	0.25-0.5	≤0.03	0.25-8	2-4
BC-9538	0.5-1	1	1-4	0.5-1	≤0.03	0.125-1	2
BC-9545	0.5-1	1	1-2	0.5-1	≤0.03	0.06-2	2-4
BC-9543	0.25-0.5	0.5	0.5-2	0.5	≤0.03	0.5-8	2-4
BC-9556	0.25-0.5	1	1-2	0.5-2	≤0.03	0.5-16	4-8
BC-9561	0.5-2	2	2	0.5-2	≤0.03	0.25-8	2-8

Table 2. Antibacterial activity of ESP against clinical isolates

		BC-7641	BC-9074	BC-9529	BC-9539	BC-9543	BC-9545	BC-9556	BC-9561	BC-3781	Amoxicillin/ clavulanic acid	Azithromycin	Ceftriaxone	Ceftazidime	Ciprofloxacin	Doxycycline	Tigecycline	Vancomycin
E. coli [a] (*n* = 32)	MIC_{50}	2	0.12	0.5	1	0.5	0.5	0.5	1	**32**	**16**	ND	**>16**	**32**	**16**	**8**	0.25	ND
	MIC_{90}	4	0.5	1	2	0.5	1	1	2	**32**	**>32**	ND	**>16**	**>32**	**>16**	**32**	0.5	ND
K. pneumoniae [b] (*n* = 24)	MIC_{50}	2	1	1	1	1	2	2	2	**>32**	2	ND	0.06	0.25	0.03	4	2	ND
	MIC_{90}	4	2	2	2	2	4	2	4	**>32**	**>32**	ND	**>16**	**>32**	2	**>32**	4	ND
S. aureus, CA-MRSA [c] (*n* = 20)	MIC_{50}	0.12	0.25	0.06	0.03	0.12	0.06	0.25	0.12	0.06	**16**	**>32**	ND	ND	1	0.5	0.06	1
	MIC_{90}	0.12	0.25	0.06	0.06	0.12	0.06	0.25	0.12	0.06	**16**	**>32**	ND	ND	**4**	0.5	0.06	1
S. pneumoniae [d] (*n* = 30)	MIC_{50}	0.5	0.5	0.12	0.12	0.25	0.06	0.25	0.12	0.06	0.015	**8**	0.03	0.5	1	0.06	≤0.03	0.25
	MIC_{90}	1	0.5	0.25	0.25	0.5	0.125	0.5	0.25	0.12	0.25	**>16**	0.25	**4**	2	**8**	≤0.03	0.25

[a], *E. coli*: 66 % (21/32) ESBL producers; 28 % (9/32) CTX-M ß-lactamase producers;
[b], *K. pneumoniae*: 25 % (6/24) ESBL producers;
[c], CA-MRSA: 75% USA300, 25% USA400;
[d], *S. pneumoniae*: 76.7 % macrolide-resistant;

INTRODUCTION continued

A variety of novel ESP has been synthesised by the semi-synthetic modification of pleuromutilin (Figure 1) and tested for their activity against *Enterobacteriaceae*.

The screened bacterial species included clinical isolates and CLSI reference strains of *E. coli*, *K. pneumoniae*, *C. freundii*, *E. cloacae*, *S. aureus*, *S. pneumoniae* and *H. influenzae*. Particularly carbapenem-resistant isolates producing metallo ß-lactamases (NDM-1), KPC ß-lactamases or other ESBL were included to evaluate if ESP meet the high demand for new antibiotics being active against those pathogens.

Figure 1. ESP Structure

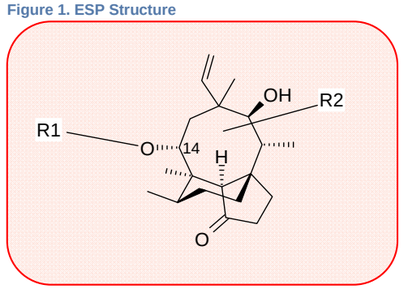

Table 3. MIC of ESP against carbapenem-resistant *Enterobacteriaceae*

Compound	NDM-1 or KPC-2/-3 producing *E. coli* (*n* = 2)	*K. pneumoniae* (*n* = 8)	*E. cloacae* (*n* = 2)
Meropenem [a]	**32**	**0.5->32**	**>32**
Ceftriaxone [a]	**>32**	**>32**	**>32**
Ceftazidime [a]	**>32**	**>32**	**>32**
Ciprofloxacin [a]	**>32**	**>32**	**>32**
Doxycycline [a]	**8-32**	**16-32**	**32**
Tigecycline [a]	0.25	1-2	**8**
BC-7641	1-2	1-4	4-8
BC-9074	0.25	0.5-2	2-4
BC-9529	0.5	1-4	4
BC-9539	1	0.5-4	2-4
BC-9543	0.5	0.5-2	2
BC-9545	1	1-4	4
BC-9556	0.5-1	0.5-4	4
BC-9561	1-2	1-8	8

[a] tested only against the NDM-1 panel ATCC MP-18™;

RESULTS

- The novel ESP displayed potent antibacterial activity against the screened bacterial isolates (Table 1) with MICs ranging between 0.12 and 2 µg/mL against *E. coli,* the major causative agent of urinary tract infections. ESP also covered *K. pneumoniae*, *C. freundii* and *E. cloacae* and maintained the activity against the species covered by earlier generation pleuromutilins, including *S. aureus*, *S. pneumoniae* and *H. influenzae.*
- When tested against larger panels of clinical *E. coli*, *K. pneumoniae*, MRSA and *S. pneumoniae* isolates ESP appeared to be as potent as tigecycline, while resistance rates among the other tested antibiotics were high (Table 2). $MIC_{50/90}$ of ESP were largely in agreement with the MICs obtained in the screening.
- ESP were fully active against fluoroquinolone- and doxycycline-resistant and ESBL producing *E. coli* and *K. pneumoniae*, macrolide-resistant MRSA and penicillin- or macrolide- resistant *S. pneumoniae* (Table 2).
- Among the tested ESP, BC-9529, BC-9540, BC-9543 and BC-9556 appeared to be the most active derivatives and further evaluation of these compounds is warranted.
- ESP displayed potent activity against carbapenem-resistant *Enterobacteriaceae* producing KPC-2/-3 ESBL or NDM-1 metallo ß-lactamase (Table 3).

CONCLUSIONS

- The novel ESP demonstrated potent activity against the most prevalent Gram-positive and Gram-negative organisms including multi-drug and carbapenem-resistant isolates.
- The additional coverage of *Enterobacteriaceae* represents a significant extension of the antibacterial profile of conventional pleuromutilins.
- Thus, further development of ESP, the new generation of pleuromutilin antibiotics, might lead to additional treatment options for patients with infections caused by multi-drug resistant bacteria such as carbapenem-resistant *E. coli* and *K. pneumoniae.*

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