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In Vitro and *In Vivo* Efficacy of Novel Extended Spectrum Pleuromutilins Against *S. aureus* and *S. pneumoniae*

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ABSTRACT

Objective: Resistance development to antibiotics imposes a growing public health threat worldwide. In Europe particularly the resistance of *E. coli* and *K. pneumoniae* against cephalosporins, fluoroquinolones and aminoglycosides increased significantly while the percentage of MRSA was decreasing during the last few years. Nevertheless, MRSA remains above 25 % in almost one fourth of the reporting countries and is one of the most frequent causes of antibiotic-resistant healthcare-associated infections worldwide (CDC, 2013). Thus the need for new antibiotics acting against those pathogens is evident.
The recently discovered extended spectrum pleuromutilins (ESP) address this problem by being active against Gram-negative and -positive bacteria. This study investigated the *in vitro* activity of four novel ESP derivatives against *S. aureus* (MSSA, MRSA) and *S. pneumoniae* including multi-resistant isolates. Furthermore, the ESP derivatives were evaluated in an initial *in vivo* efficacy model in mice infected with *S. aureus* to proof their therapeutic potential in comparison to linezolid and tigecycline.
Methods: MIC of ESP and comparators against MSSA (*n* = 25), CA-MRSA (*n* = 20) and *S. pneumoniae* (*n* = 32) were determined by broth microdilution according to CLSI (M7/A9). To evaluate the therapeutic potency of the selected ESP *in vivo*, mice were infected intraperitoneally with an inoculum of approximately 4 x 10^7 CFU MSSA per mouse causing a lethal sepsis within 24 h. The drugs were administered s.c. as single dose 1 h post infection and survival was recorded for 96 h. The total daily dose required for survival of 50 % of mice (ED_{50}) and 95 % confidence limits were calculated by binary probit analysis.
Results: The tested ESP derivatives BC-7634, BC-9074, BC-9529, and BC-9563 showed potent antibacterial activity against MSSA and CA-MRSA including predominantly USA300 strains with MIC_{90} of ≤ 0.25 µg/mL. Against *S. pneumoniae* ESP displayed MIC_{90} of 0.25-0.5 µg/mL. The activity was unaffected by resistance to macrolides, tetracyclines, or cephalosporins.
In the *S. aureus* bacteremia model all selected ESPs showed good *in vivo* efficacy when compared to linezolid and tigecycline. BC-7634, BC-9074, BC-9529, and BC-9563 showed ED_{50} values of 0.26 mg/kg/day, 0.57 mg/kg/day, 0.47 mg/kg/day and 0.14 mg/kg/day, respectively. Linezolid and tigecycline had ED_{50} values of 10.3 mg/kg/day and 0.99 mg/kg/day, respectively.
Conclusion: The novel ESPs showed potent activity against *S. aureus* (MSSA and CA-MRSA) and *S. pneumoniae*. In a murine bacteremia caused by *S. aureus* all tested ESP showed good efficacy being as active as tigecycline and significantly more active than linezolid. These proof-of-concept studies warrant the further development of ESP since the antibacterial spectrum does not only cover resistant *Enterobacteriaceae* but ESP additionally demonstrate potent activity against Gram-positive pathogens.

INTRODUCTION

Extended spectrum pleuromutilins (ESP) are a new generation of semi-synthetic pleuromutilin derivatives. By the substitution at the C-14 side chain and the tricyclic pleuromutilin core (Figure 1) broad-spectrum activity was achieved against a wide range of Gram-negative and Gram-positive bacterial pathogens including *Enterobacteriaceae*, staphylococci, streptococci and fastidious Gram-negative bacteria. ESP are potent inhibitors of the bacterial protein translation by specific binding to the peptidyl transferase center and thereby overcome major resistances.[1, 2, 3]

Four novel ESP derivatives from Nabriva's drug discovery program have been selected for this study based on their potent *in vitro* activity against *E. coli*, low potential for cytotoxicity and high metabolic stability[4] to investigate the antibacterial activity of these ESP derivatives against larger sets of *S. aureus* (MSSA), community-acquired methicillin-resistant *S. aureus* (CA-MRSA) and *S. pneumoniae* in addition to *E. coli*. Furthermore, the *in vivo* activity against *S. aureus* was explored in a bacteremia model in mice.

METHODS

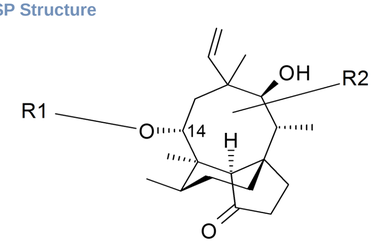
MIC determination and bacterial isolates

The minimal inhibitory concentration (MIC) was determined by broth microdilution using CA-MHB according to CLSI M7-A9 (2012).
Bacterial strains were kindly provided by various sources: MSSA isolates by ATCC and the general hospital (AKH) Vienna, Austria; CA-MRSA and *S. pneumoniae* by JMI Laboratories (North Liberty, IA, USA); *E. coli* isolates by D. Livermore (Health Protection Agency, UK) and F.J. Schmitz (Klinikum Minden, D).

In vivo bacteremia model in mice

The *in vivo* antibacterial activity of the ESP and tigecycline was determined in a sepsis model in immuno-competent mice. NMRI mice were infected intraperitoneally with *S. aureus* ATCC49951 using an inoculum of approximately 4 x 10^7 CFU per mouse. The drugs were administered s.c. as single dose 1 h post infection and survival was recorded for 96 h. The total daily dose required for survival of 50 % of mice (ED_{50}) and 95 % confidence limits were calculated by binary probit analysis.

Figure 1. ESP Structure



RESULTS

- All selected ESP (BC-7634, BC-9563, BC-9529 and BC-9563) having distinct substitutions at R1 and R2 (Figure 1) exhibited potent antibacterial activity against the tested clinical *S. aureus* isolates including both MSSA and CA-MRSA (Table 1).
- The MIC_{90} of ESP against CA-MRSA including isolates resistant to macrolides, tetracyclines and fluoroquinolones ranged between ≤0.03 and 0.25 µg/mL, which was as potent as tigecycline (MIC_{90}, 0.06 µg/mL) and significantly more active than vancomycin (MIC_{90}, 1 µg/mL).
- Against *S. pneumoniae* the ESP derivatives were with MIC_{90} of 0.25 - 0.5 µg/mL similarly potent as ceftriaxone (MIC_{90}, 0.25 µg/mL) and vancomycin (MIC_{90}, 0.25 µg/mL, Table 1).
- No cross-resistance was observed with macrolides, tetracyclines, ß-lactam antibiotics or fluoroquinolones.
- In the murine bacteremia model all selected ESP showed good *in vivo* efficacy with all ESP displaying ED_{50} of 0.14-0.57 mg/kg (Table 2).
- Overall, the ED_{50} correlated well with the *in vitro* activity.
- The ED_{50} of the tested ESP were comparable to that of tigecycline (ED_{50} of 0.99 mg/kg) and significantly more active than linezolid (ED_{50} of 10.3 mg/kg).

Table 1. Antibacterial activity [µg/mL] of novel ESP derivatives against clinical *S. aureus* and *S. pneumoniae* isolates

		BC-7634	BC-9074	BC-9529	BC-9563	Amoxicillin/ Clavulanic acid	Azithromycin	Ceftriaxone	Ceftazidime	Ciprofloxacin	Doxycycline	Tigecycline	Vancomycin	Linezolid
***S. aureus*, MSSA** (*n* = 25)	MIC_{50}	0.06	0.12	0.03	0.03	0.12	0.5	4	8	0.25	0.06	ND	1	2
	MIC_{90}	0.12	0.25	0.12	0.06	0.25	>32	4	16	1	0.25	ND	1	4
S. aureus, CA-MRSA [a] (*n* = 20)	MIC_{50}	0.12	0.25	0.06	≤0.03	**16**	**>32**	ND	ND	1	0.5	0.06	1	2
	MIC_{90}	0.12	0.25	0.06	≤0.03	**16**	**>32**	ND	ND	**4**	0.5	0.06	1	4
S. pneumoniae [b] (*n* = 30)	MIC_{50}	0.25	0.5	0.12	0.25	0.015	**8**	0.03	0.5	1	0.06	≤0.03	0.25	1
	MIC_{90}	0.25	0.5	0.25	0.5	0.25	**>16**	0.25	**4**	2	**8**	≤0.03	0.25	1
E. coli [c] (*n* = 32)	MIC_{50}	0.5	0.12	0.5	1	**16**	ND	**>16**	**32**	**16**	**8**	0.25	ND	ND
	MIC_{90}	1	0.5	1	1	**>32**	ND	**>16**	**>32**	**>16**	**32**	0.5	ND	ND

[a], CA-MRSA: 75 % USA300, 25 % USA400; [b], *S. pneumoniae*: 76.7 % macrolide-resistant; [c], *E. coli*: 28.1% CTX-M ß-lactamase producers, 50% TEM-type ß-lactamase producers

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Table 2. *In vivo* efficacy and antibacterial activity of ESP and comparators against *S. aureus* ATCC 49951

Compound	MIC [mg/L]	ED_{50} [mg/kg/day]
BC-7634	≤0.03	0.26
BC-9074	0.06	0.57
BC-9529	≤0.03	0.47
BC-9563	≤0.03	0.14
Linezolid	2	10.3
Tigecycline	0.25	0.99

CONCLUSIONS

- **The presented extended spectrum pleuromutilins (ESP) having various distinct side chains at R1 and R2 demonstrated potent *in vitro* activity against staphylococci and streptococci including CA-MRSA and resistant clinical isolates.**
- **ESP maintained their activity against Gram-positive organisms while their antibacterial spectrum was extended by Enterobacteriaceae.**
- **The good *in vitro* antibacterial activity against *S. aureus* could be fully translated into good *in vivo* activity in the bacteremia model in mice demonstrating good drug disposition.**
- **Based on the data presented, ESP will be further explored for development as potent broad-spectrum antibiotics.**

REFERENCES

(1) Paukner S., Strickmann D., Ivezic-Schoenfeld Z. Extended Spectrum Pleuromutilins: Mode-of-Action Studies. *24th ECCMID,* Barcelona, E. Poster P1681 (2014)

(2) Paukner S., Kollmann H., Thirring K., Heilmayer W., Ivezic-Schoenfeld Z. Antibacterial *in vitro* activity of novel extended spectrum pleuromutilins against Gram-positive and –negative bacterial pathogens. *24th ECCMID,* Barcelona, E. Poster P1678 (2014)

(3) Wicha W.W., Ivezic-Schoenfeld Z. *In vivo* activity of extended spectrum pleuromutilins in murine sepsis model. *24th ECCMID,* Barcelona, E. Poster P1680 (2014)

(4) Wicha W.W., Paukner S., Strickmann D.B., Thirring K., Kollmann H., Heilmayer W., Ivezic-Schoenfeld Z. Efficacy of novel ESP against *E. coli in vitro* and *in vivo*. *25th ECCMID*, Copenhagen, DK. E-poster EV0208 (2015)

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