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Kill curves of the Novel Extended-Spectrum Pleuromutilin Antibiotic BC-9529

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ABSTRACT

Objective: Extended spectrum pleuromutilins (ESP) are the second generation of pleuromutilin antibiotics which exhibit potent activity against Gram-negative pathogens like *Escherichia coli*, *Klebsiella pneumoniae* and other Enterobacteriaceae along with staphylococci, streptococci, *Haemophilus influenzae* and atypical respiratory pathogens. The coverage of highly resistant pathogens including carbapenem-resistant and ESBL producing Enterobacteriaceae, MDR *Streptococcus pneumoniae* and MRSA makes ESP an attractive treatment option in the fight against the dramatic increase and rapid spread of multi-drug resistances.[1-4] This study investigated the antibacterial activity and bactericidal properties of the novel investigative ESP derivative BC-9529 against *E. coli*, *K. pneumoniae* and *S. aureus*.

Methods: The antibacterial activity was determined by broth microdilution (CLSI, M7-A9) against *E. coli* (*n* = 32) including ESBL (TEM, CTX-M) producing strains (78.1 %), *K. pneumoniae* (*n* = 24; 25.0 % ESBL producing) and *S. aureus* (CA-MRSA, *n* = 20; 100% macrolide-resistant). Kill curves were determined for three *E. coli*, one *K. pneumoniae* and one *S. aureus* strain by broth macrodilution in CAMHB for BC-9529 at 1- to 16-fold MIC in comparison to moxifloxacin and tigecycline.

Results: The novel ESP derivative BC-9529 showed potent antibacterial activity against the tested *E. coli* ($MIC_{50/90}$ 0.5/1 µg/mL), *K. pneumoniae* ($MIC_{50/90}$, 1/2 µg/mL), and *S. aureus* ($MIC_{50/90}$, 0.06/0.06 µg/mL). It was fully active against TEM, CTX-M, NDM-1 or KPC producing strains which were largely resistant to ß-lactam antibiotics, ciprofloxacin and doxycycline.
Furthermore, BC-9529 demonstrated bactericidal activity against *E. coli* and *K. pneumoniae* whereas it appeared to be bacteriostatic against *S. aureus*. Living cell counts of *E. coli* (including a CTX-M 15 producing strain) were reduced by at least 4 log_{10} at ≥ 2-fold MIC (corresponding to ≥ 1 µg/mL) within 24 h, that of *K. pneumoniae* by > 3 log_{10} at ≥ 8-fold MIC. Killing of *E. coli* and *K. pneumoniae* was generally rapid (within 6 h of incubation) and dependent on time and concentration. Against *S. aureus* BC-9529 was bacteriostatic with living cell count reductions of ~1-2 log_{10} within 24 h. Overall, the potent bactericidal effect of BC-9529 on *E. coli* was comparable to that of moxifloxacin. Killing of *E. coli* by tigecycline was slower and time-dependent (T_{max} = 24 h). Against *K. pneumoniae* and *S. aureus* tigecycline exhibited a bacteriostatic effect (> 1.5 log_{10} within 24 h).

Conclusion: The novel ESP BC-9529 demonstrated good antibacterial activity against highly resistant bacterial pathogens including ESBL and carbapenemase-producing Enterobacteriaceae and CA-MRSA. The high antibacterial and bactericidal activity against both, *E. coli* and *K. pneumoniae* are attractive qualities in the overall potent antibacterial profile, which might lead to potential future treatment options against serious infections caused by these pathogens.

RESULTS

- BC-9529 demonstrated potent antibacterial activity against clinical isolates of *E. coli*, *K. pneumoniae* and MRSA (Table 1).
- The MIC_{90} against *E. coli and K. pneumoniae* including a high proportion of resistant isolates was 1 µg/mL and 2 µg/mL, which was as active as tigecycline (MIC_{90}, 0.5 and 4 µg/mL, respectively). Against *S. aureus* (MRSA) BC-9529 was with a MIC_{90} of 0.06 µg/mL even more potent.
- BC-9529 was bactericidal against *E. coli* and *K. pneumoniae* with living cell count reductions of > 3 log_{10} within 6 h at ≥ 2x MIC for *E. coli* ATCC29522 and *E. coli* D120. Against the CTX-M producing *E. coli* B1098 and *K. pneumoniae* ATCC 43816 killing by BC-9529 was slower and more dependent on the drug concentration (Figure 1 and Table 2).

RESULTS continued

- Overall, killing of Enterobacteriaceae by BC-9529 was rapid and dependent on time and BC-9529 concentration. Reduction of living cell counts by BC-9529 was more pronounced and faster than by tigecycline and as fast as moxifloxacin known to act bactericidal.
- Against *S. aureus* BC-9529 was bacteriostatic with living cell count reductions of approx. 2 log_{10} within 24 h. This was comparable to the effect of tigecycline.
- To investigate the reason for the bactericidal activity, initial membrane depolarization and permeabilisation experiments were performed using $Disc_3(5)$ and Syto9/propidium iodide dyes. Results showed that BC-9529 did not depolarize the *E. coli* membrane and indicated an effect of BC-9529 on the membrane permeability of *E. coli*.

Figure 1. Time-kill curves of BC-9529, tigecycline and moxifloxacin

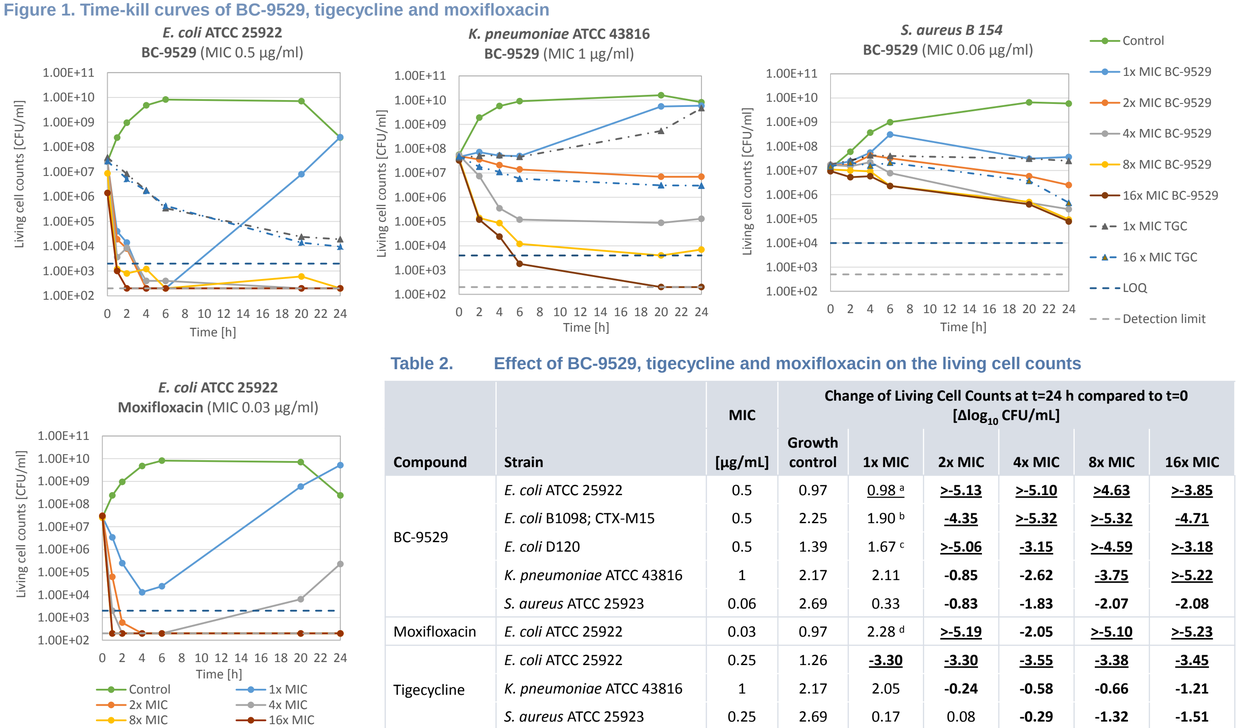

Table 2. Effect of BC-9529, tigecycline and moxifloxacin on the living cell counts

Compound	Strain	MIC [µg/mL]	Change of Living Cell Counts at t=24 h compared to t=0 [Δlog$_{10}$ CFU/mL] Growth control	1x MIC	2x MIC	4x MIC	8x MIC	16x MIC
BC-9529	*E. coli* ATCC 25922	0.5	0.97	0.98 [a]	**>-5.13**	**>-5.10**	**>4.63**	**>-3.85**
	E. coli B1098; CTX-M15	0.5	2.25	1.90 [b]	**-4.35**	**>-5.32**	**>-5.32**	**-4.71**
	E. coli D120	0.5	1.39	1.67 [c]	**>-5.06**	**-3.15**	**>-4.59**	**>-3.18**
	K. pneumoniae ATCC 43816	1	2.17	2.11	**-0.85**	**-2.62**	**-3.75**	**>-5.22**
	S. aureus ATCC 25923	0.06	2.69	0.33	**-0.83**	**-1.83**	**-2.07**	**-2.08**
Moxifloxacin	*E. coli* ATCC 25922	0.03	0.97	2.28 [d]	**>-5.19**	**-2.05**	**>-5.10**	**>-5.23**
Tigecycline	*E. coli* ATCC 25922	0.25	1.26	**-3.30**	**-3.30**	**-3.55**	**-3.38**	**-3.45**
	K. pneumoniae ATCC 43816	1	2.17	2.05	**-0.24**	**-0.58**	**-0.66**	**-1.21**
	S. aureus ATCC 25923	0.25	2.69	0.17	0.08	**-0.29**	**-1.32**	**-1.51**

Bold, reduction of living cell counts compared to t = 0 h; **Bold and underlined**, reduction of living cell counts > 3 log_{10}
[a-d], Regrowth at t=24 h; maximum CFU reduction for [a] >-5.11 log_{10} at t=4 h, [b] -2.15 log_{10} at t=6 h, [c] -1.54 log_{10} at t=6 h and [d] -3.32 log_{10} at t=4 h.

Table 1. Antibacterial activity of BC-9529 and comparators

Species		BC-9529	Ciprofloxacin	Tigecycline
E. coli [a]	MIC_{50}	0.5	16	0.25
(*n* = 32)	MIC_{90}	1	>16	0.5
K. pneumoniae [b]	MIC_{50}	1	0.03	2
(*n* = 24)	MIC_{90}	2	2	4
***S. aureus*, MRSA** [c]	MIC_{50}	0.06	1	0.06
(*n* = 20)	MIC_{90}	0.06	4	0.06

[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

CONCLUSIONS

- **The novel ESP derivative BC-9529 demonstrated potent antibacterial activity against Gram-negative and Gram-positive bacterial pathogens which cause serious infections and show an alarming trend in resistance development.**
- **BC-9529 displayed rapid bactericidal activity against *E. coli* and *K. pneumoniae*. Against *S. aureus* the ESP BC-9529 remained bacteriostatic similar to the 1st generation pleuromutilins.**
- **This bactericidal activity of ESP represents an interesting feature for the treatment of bacterial infections caused by Enterobacteriaceae.**
- **Additional studies will be conducted to further explore the mode-of-action of this novel generation of pleuromutilin 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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