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Macrolones - Novel Class of Macrolide Antibiotics Active Against Key Resistant Respiratory Pathogens

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Introduction

Respiratory tract infections (RTI) are among the most common illnesses worldwide, resulting in high costs of care, absence from work, decreased productivity and high antibiotic consumption. Predominant bacterial pathogens are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes* and atypicals (e.g. *Legionella pneumophyla*, *Mycoplasma pneumoniae*) while *Staphylococcus aureus* incidence is rising. For community acquired pneumonia (CAP), mortality rates vary, but can be as high as 30% among ICU patients, and *S. pneumoniae* is the most prevalent pathogen in CAP (1;2). Dissemination of multi-resistant strains is of great concern. Major mechanisms of erythromycin resistance are ribosome methylation (MLSb phenotype) and production of efflux pumps (M phenotype). Pneumococcal isolates with mutations in 23S rRNA and ribosomal proteins L4 and L22 have been reported, but their clinical significance is still rather low (3).

We have reported on the synthesis and activity of a novel macrolide antibiotic class named "macrolones" (4-6), with an improved spectrum of activity covering resistant strains of predominant RTI pathogens. Here we present the activity and mode of action of representative compounds, including *in vivo* efficacy results from the mouse pneumonia model induced by eryS and cMLSb strains of *S. pneumoniae*, as well as the pharmacokinetics (PK) in mice.

Methods

Antibacterial activity was determined by broth microdilution according to CLSI guidelines (7) and *in vitro* pharmacodynamics assessed using time-kill methodology.

For macromolecular synthesis (MMS), incorporation of radioactively labeled precursors of major metabolic pathways was monitored. The transcription/translation (TnT) assay was done using an *in vitro* protein synthesis kit *E. coli* S30 Extract System for Circular DNA (Promega). *In vitro* resistance development was assessed by measuring spontaneous mutation rates and by passage studies in sub-inhibitory concentrations of compounds. In mutants, genes for 23S rRNA domains II and V and ribosomal proteins L4 and L22 were sequenced.

In vivo pneumonia in male C57Bl6J mice was induced intranasally (i.n.) with 10^6 CFU/50mL PBS. Compounds where applied orally (p.o.) or intarperitoneally (i.p.), once or twice daily. Animals were sacrificed 44h p.i., and CFU counts in lung homogenates determined. Statistical analyses was done by ANOVA, Kruskal-Wallis test. PK studies were done in male Balb/c mice following intravenous (i.v.) bolus and p.o. dosing. Blood samples were analyzed using LC-MS/MS and PK parameters are reported as mean values ±SD.

Results

Macrolones are a novel class of antibacterial macrolide compounds, characterized by a quinolone moiety attached via linker by an ester bond to 4" position of macrolide scaffold. General formula and structure of representative compound 7 are given in Figure 1. The spectrum of activity for macrolones covers key bacterial RT pathogens with *in vitro* profiles superior to those of marketed macrolide antibiotics and telithromycin (TEL), including eryR *S. pneumoniae*, *S. pyogenes* and *S. aureus* strains (Table 1). Compound 7 has MIC_{90} values against MLSb *S. pneumoniae* and *H. influenzae* comparable to TEL, and is superior against iMLSb and cMLSb strains of *S.pyogenes* (MIC_{90} ≤0.015 vs. 4 and 0.5 vs. 32 µg/mL, respectively).

Unlike macrolides and ketolides, macrolones showed rapid bactericidal effects against *H. influenzae* (Figure 2), eradicating bacteria below the detection limit after 2h of incubation at 4xMIC. Their activity at the ribosomal level was confirmed by the TnT assay, where they show IC_{50} values in the nanomolar range comparable to AZI, ERY and TEL (Table 3), as well as by the MMS assays, showing most significant inhibition on protein synthesis (Figure 3). Macrolones exhibited equal or lower *in vitro* resistance development potential than AZI and TEL in *S. pneumoniae*, while in *H.influenzae*, *S.aureus* and *M.catarrhalis* mutants were not induced (Table 4). In the *S. pneumoniae* passage study, TEL and compound 7 mutants emerged after 21 and 43 days, respectively. Mutations were found in the gene for ribosomal protein L22 and represented point mutations or 8 amino-acid duplication and insertion between P82 and R83 (Table 5).

The *in vitro* efficacy was confirmed in the murine pneumonia model induced by both eryS and eryR *S. pneumoniae*. After i.p. dosing, compounds 3 and 7 cleared both strains from lungs below the detection limit. Macrolones have a low clearance, large volume of distribution, and long half-life, complying with once-daily dosing potential (Table 6).

Results

Table 1. Summary data of antibacterial activity of novel macrolone compounds against key respiratory pathogens, grouped by species and resistance mechanism, given as MIC_{90} values in µg/mL.

		S.pneumoniae			*S. pyogenes*				*S. aureus*			*H. inf.*
cpd	Phenotype	eryS	M	MLSb	eryS	M	iMLSb	cMLbS	eryS	M	iMLSb	
	No. isolates	10	13	13	10	12	14	13	11	10	12	12
AZI	Azithromycin	0.03	8	>64	0.06	4	>64	>64	1	>64	>64	2
CLA	Clarithromycin	0.03	8	>64	≤0.015	4	>64	>64	0.125	32	>64	16
TEL	Telithromycin	≤0.015	0.5	0.5	≤0.015	0.5	4	32	0.06	0.25	0.06	2
CLI	Clindamycin	≤0.015	0.03	>64	0.03	0.03	0.3	>64	0.06	0.125	0.125	32
CIP	Ciprofloxacin	0.5	1	1	0.25	0.5	1	0.5	>64	0.5	>64	≤0.015
1	azi-NN-Cl	0.125	0.5	0.125	0.06	1	0.25	0.5	2	1	2	8
2	azi-NMeN-Cl-Qme	≤0.015	0.06	0.06	0.06	0.03	0.25	1	4	16	32	16
3	azi-ON-Cl	≤0.015	≤0.015	0.125	≤0.015	0.03	≤0.015	0.25	0.5	0.5	0.5	2
4	azi-ON	≤0.015	0.06	1	≤0.015	0.06	0.03	1	0.5	1	1	2
5	clari-ON-Cl	≤0.015	≤0.015	0.25	≤0.015	≤0.015	0.03	0.5	0.5	0.5	1	4
6	azi-OO-Cl	≤0.015	≤0.015	0.5	≤0.015	0.03	≤0.015	1	0.5	1	0.5	2
7	azi-OON-Cl	≤0.015	≤0.015	0.25	≤0.015	≤0.015	≤0.015	0.5	0.5	0.5	0.5	2
8	azi-OON	≤0.015	0.03	0.5	≤0.015	0.06	≤0.015	0.25	0.25	0.25	0.25	8
9	clari-OON-Cl	≤0.015	0.06	4	0.03	0.125	2	2	1	2	8	8
10	claric-OON-Cl	≤0.015	≤0.015	0.125	≤0.015	≤ 0.03	≤0.015	0.25	0.25	0.5	1	4
11	8a-OON-Cl	≤0.015	≤0.015	0.125	≤0.015	0.03	0.03	0.5	0.25	0.25	0.25	4
12	eox-OON-Cl	≤0.015	≤0.015	0.5	≤0.015	≤0.015	≤0.015	0.5	0.25	0.25	0.5	2
13	rox-OON-Cl	0.06	0.125	2	0.06	0.06	0.25	2	0.5	1	4	4

Table 2. MIC_{90} values of cpd 7 against recent RTI isolates of *S. pneumoniae* and *H. influenzae* from Croatia, given in µg/mL.

Compound	*S. pneumoniae* (n=73)	*H. influenzae* (n=37)
7	≤0.015	4
TEL	0.125	2
AZI	>64	2
CLI/CLA*	>64	8
TET	32	16
PEN/AMP*	2	1
CTX	1	≤0.03
MOX	0.125	≤0.03

Table 3. Inhibition of prokaryotic protein synthesis, determined by *E. coli* TnT assay, given as IC_{50} values in µM.

	Compound	IC_{50} (µM)
1	azi-NN-Cl	0.28
3	azi-ON-Cl	0.31
5	clari-ON-Cl	0.86
6	azi-OO-Cl	0.58
7	azi-OON-Cl	0.19
TEL	Telithromycin	0.31
AZI	Azithromycin	0.32
ERY	Erythromycin	0.45
CIP	Ciprofloxacin	>50

Table 4. Rates of spontaneous resistance development to macrolone 7, AZI and TEL against four bacterial species.

cpd	Strain	MIC	4xMIC	10xMIC
7	*S. pneumoniae* SP030	0,06	$5x10^{-10}$	$<5x10^{-10}$
	H. influenzae H128	2	$<3x10^{-9}$	$<3x10^{-9}$ x
	H. infl. ATCC49247	2	$<1x10^{-10}$	$<1x10^{-10}$
	M. catarrhalis Ravasio	0.25	$<6x10^{-9}$	$<6x10^{-9}$
	S. aureus WCUH29	0.5	$<5x10^{-9}$	$<5x10^{-9}$
AZI	*S. pneumoniae* SP030	0.06	$<5x10^{-10}$	$<5x10^{-10}$
	H. influenzae H128	0.25	$<2x10^{-9}$	$<2x10^{-9}$
	H. infl. ATCC49247	1	$1x10^{-10}$	$<1x10^{-10}$
	M. catarrhalis Ravasio	0.03	$<6x10^{-9}$	$<6x10^{-9}$
	S. aureus WCUH29	0.5	$<1x10^{-9}$	$<1x10^{-9}$
TEL	*S. pneumoniae* SP030	0,03	$5x10^{-10}$	$<5x10^{-10}$
	H. influenzae H128	1	$<3x10^{-9}$	$<3x10^{-9}$
	H. infl. ATCC49247	2	$3x10^{-10}$	$<1x10^{-10}$
	M. catarrhalis Ravasio	0.125	$<6x10^{-9}$	$<6x10^{-9}$
	S. aureus WCUH29	0.125	$<5x10^{-9}$	$<5x10^{-9}$

Table 5. Changes in protein L22 of *S. pneumoniae* mutants induced by macrolone 7, AZI and TEL.

Position	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
SP 030	K	R	F	R	P	-	-	-	-	-	-	-	-	R	A	K	G
7-ss	K	R	F	R	P	R	A	K	G	S	A	S	P	I	N	K	G
TEL-ms	K	R	F	R	P	-	-	-	-	-	-	-	-	R	A	K	D
TEL ss	K	R	F	R	P	-	-	-	-	-	-	-	-	R	A	K	G
Position	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	
SP 030	S	A	S	P	I	N	K	R	T	A	H	I	T	V	A	V	
7-ss	S	A	S	P	I	N	K	R	T	A	H	I	T	V	A	V	
TEL-ms	S	A	S	P	I	N	K	R	T	A	H	I	T	V	A	V	
TEL ss	S	A	S	P	I	N	K	R	T	A	H	I	T	V	T	V	

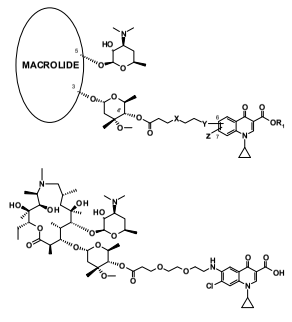

Figure 1. a) General formula of macrolones. X=O, N or OCH2CH2; Y=O or N; Z halogen atom (Cl or F); R1 ester of quinolone carboxilic acid, b) Compound 7.

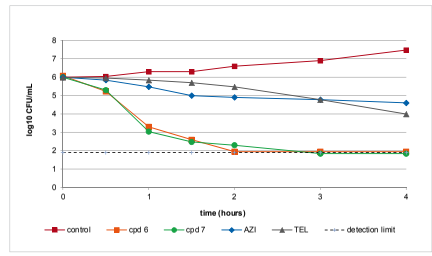

Figure 2. Effects of compounds 6, 7, AZI, and TEL on *H. influenzae* ATCC 49247 in the first 4h of incubation, at concentration of 4xMIC. MIC values in µg/mL were: cpd 6=2; cpd 7=1; AZI=1 and TEL=1.

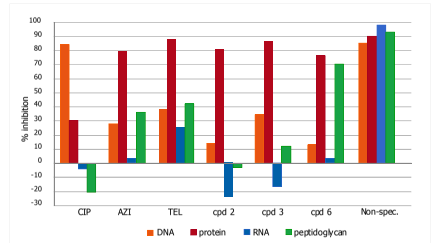

Figure 3. Effects of macrolones, AZI, TEL and CIP on macro-molecular synthesis in *S. aureus* ATCC13709.

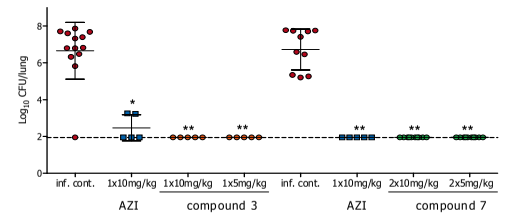

Figure 4. Efficacy of macrolones 3 and 7 after i.p. administration in mouse pneumonia caused by eryS *S. pneumoniae* SP030 strain. MICs of cpd 3, cpd 7 and AZI are ≤0.015, 0,03 and 0.03 µg/mL, respectively.

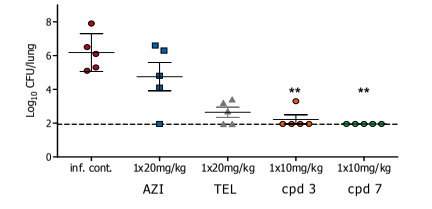

Figure 5. Efficacy of macrolones 3 and 7 after i.p. administration in mouse pneumonia caused by MLSb *S. pneumoniae* 1217 strain. MICs in µg/mL were: cpd 3 and cpd 7 ≤0,015; TEL 0.06 and AZI>64 .

Table 6. PK parameters in blood of selected macrolones in mice (3 animals per group) after intravenous and oral administration.

cpd	IV bolus Target dose 2 mg/kg			Oral Gavage Target dose 10 mg/kg					
	Cl	Vss	$T_{1/2}$	C_{max}	T_{max}	$AUC_{(0-t)}$	$AUC_{(0-inf)}$	$T_{1/2}$	F
	mL/min/kg	L/kg	h	ng/mL	h	ng.h/mL	ng.h/mL	h	%
3	26.7±4.5	15.0±8.3	8.2±2.0	10.0±8.0	2.0	58.0±73.9	98.4±141.2	8.5±12	2±2
7	17.9±2.7	8.2±1.7	7.9±0.7	8.0±3.0	2.0	42.3±7.1	47.3±5.4	2.5±0.2	<1
2	12.9±6.3	6.8±2.9	12.8±2.3	140±44	4.0	1769±60	ND	15.0±3.0	12±4

-Tmax expressed as median

Conclusions

- Macrolones are novel protein synthesis inhibitors active against key resistant RTI pathogens *in vitro*.
- They are rapidly bactericidal against *H. influenzae*.
- Macrolones are active against eryS and MLSb resistant *S. pneumoniae* in the murine pneumonia model.
- The results obtained clearly demonstrate superior activity of macrolones vs. marketed macrolides and ketolides.

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