

## **Macrolones – novel class of macrolide antibiotics active against key resistant respiratory pathogens *in vitro* and *in vivo***

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### **Supplementary material**

**Figure S1.** Structures of 4"-ester linked macrolones with two nitrogen atoms in the linker.

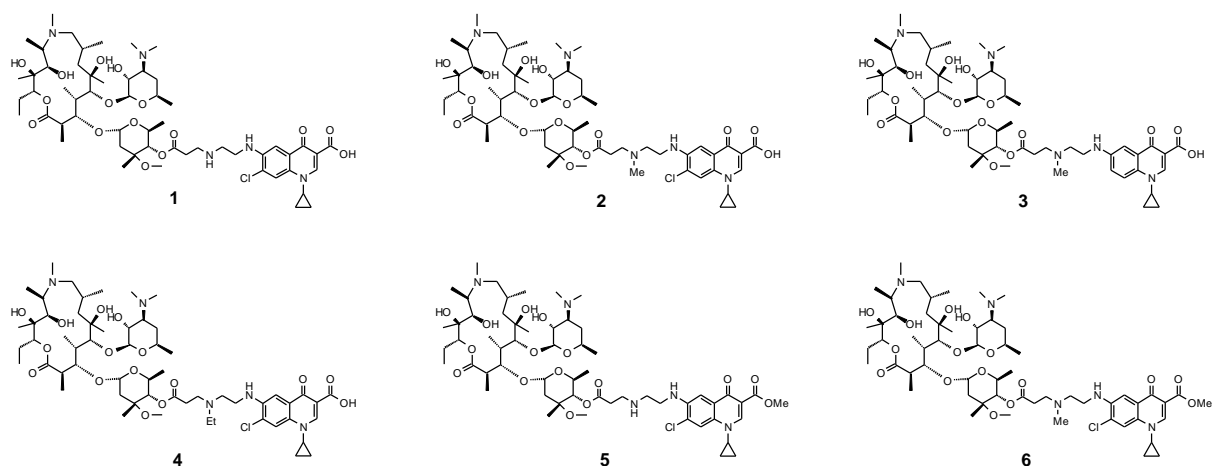
**Figure S2.** Structures of 4"-ester linked macrolones with one oxygen and one nitrogen atom in the linker.

**Figure S3.** Structures of 4"-ester linked macrolones with two oxygen atoms in the linker.

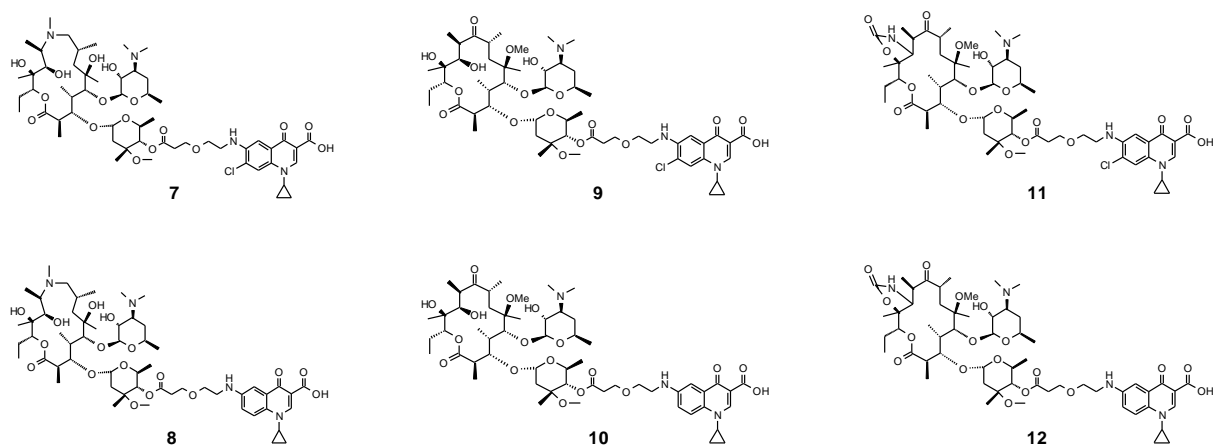
**Figure S4.** Structures of 4"-ester linked macrolones with two oxygen and one nitrogen atom in the linker.

**Figure S5.** Efficacy of compound 1 (azi-NN-Cl) in mouse pneumonia model induced by eryS *S. pneumoniae* SP030 strain.

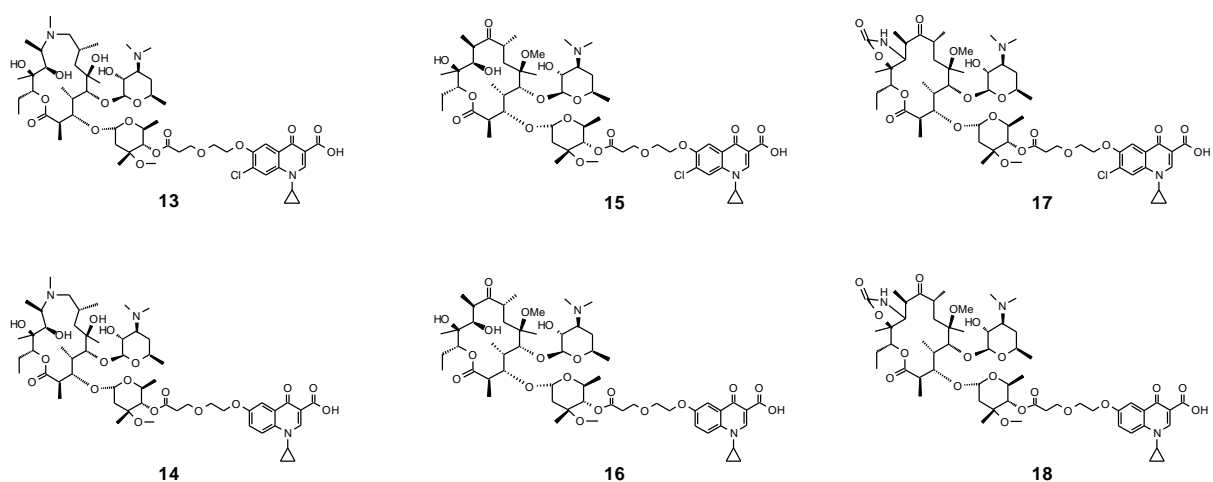
**Figure S6.** Efficacy of compound 7 (azi-NO-Cl) in mouse pneumonia model induced by eryS *S. pneumoniae* SP030 strain.



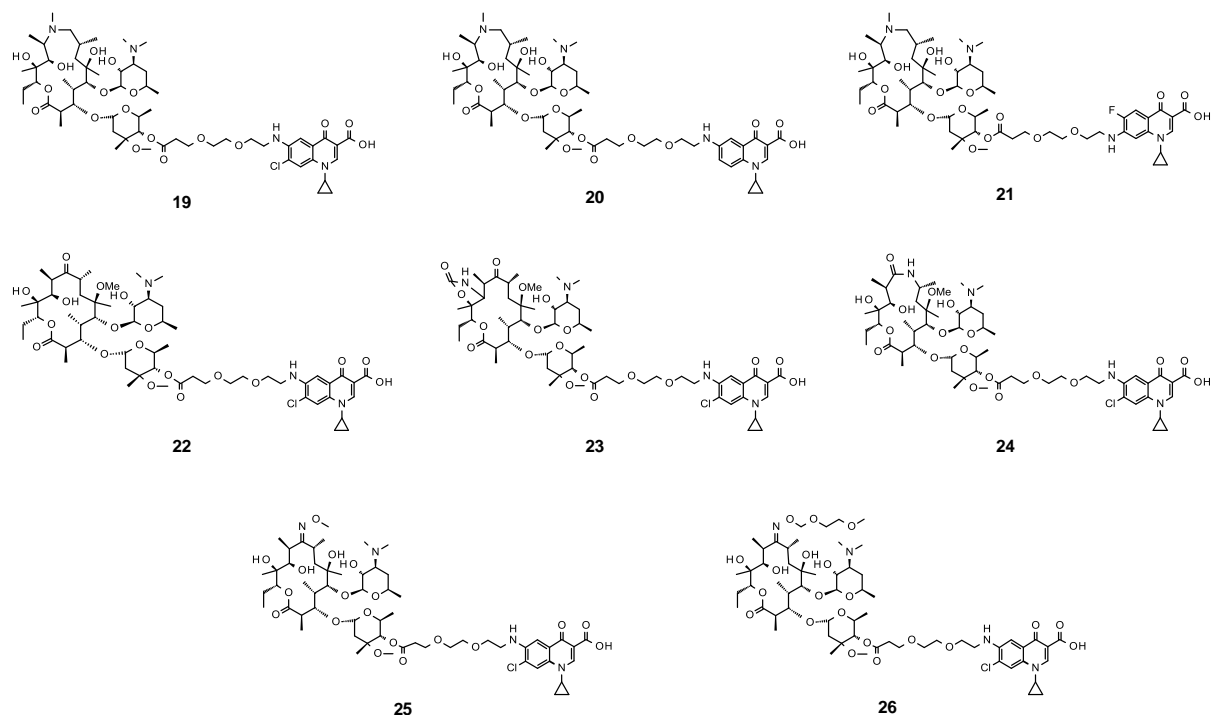
**Figure S1.** Structures of 4''-ester linked macrolones with two nitrogen atoms in the linker. Compounds 1,2,3,5,and 6 were first reported in (1) as compounds 48, 55, 58, 50 and 57, respectively, and compound 4 in (2).



**Figure S2.** Structures of 4''-ester linked macrolones with one oxygen and one nitrogen atom in the linker. Compounds 7 and 8 were first reported in (3) as compounds 24 and 32, respectively. Compounds 9 – 12 were disclosed in (4) as examples 36, 39, 30 and 31, respectively.

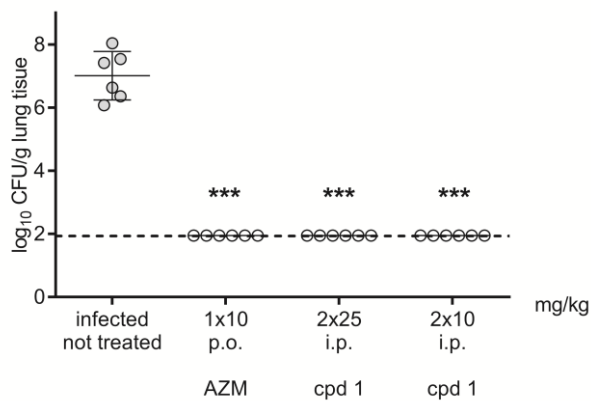


**Figure S3.** Structures of 4''-ester linked macrolones with two oxygen atoms in the linker. Compounds 13 and 14 were first reported in (3) as compounds 25 and 31, respectively. Compounds 15 – 18 were disclosed in (4) as examples 37, 38, 32 and 33, respectively.

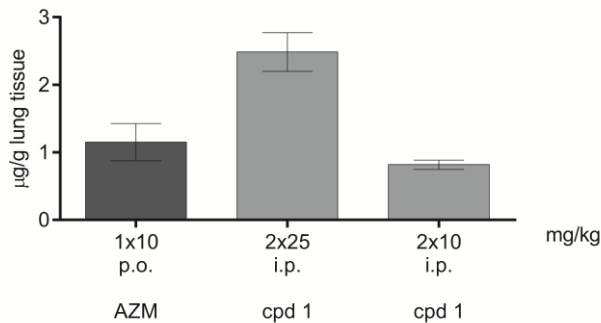


**Figure S4.** Structures of 4''-ester linked macrolones with two oxygen and one nitrogen atom in the linker. Compounds 19 and 20 were described in (3) as 29 and 33, respectively. Compounds 21, 22, 23, 24 and 26 are disclosed as examples 25, 7, 26, 64 and 25 from (5), while compound 25 is covered by general formula.

A

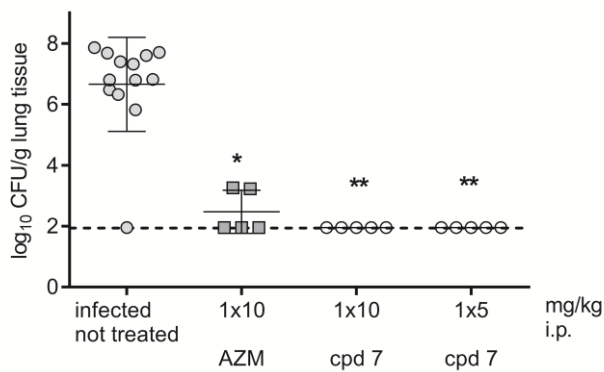


B

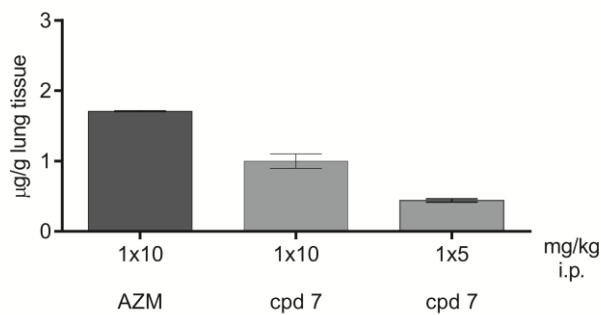


**Figure S5.** Efficacy of compound 1 (azi-NN-Cl) in mouse pneumonia model induced by eryS *S. pneumoniae* SP030 strain. Compound was administered twice daily (overall three doses of 10 or 25 mg/kg at 6, 18 and 30 h post infection) i.p. and AZM p.o. once daily (overall 2 doses of 10 mg/kg at 6 and 30 h p.i.). MIC values of compound 1 and azithromycin were 0.03 µg/ml. (a) CFU count per gram of lung tissue. Statistically significant reduction in CFU is designated by asterisk (\*\*=p<0.001; \*\*\*=p<0.0001). (b) Concentration of compound in µg/g of lung tissue 14h after the last dose, given as mean values ±SEM.

A



B



**Figure S6.** Efficacy of compound 7 (azi-ON-Cl) in mouse pneumonia model induced by eryS *S. pneumoniae* SP030 strain. Compound and azithromycin were administered i.p. once daily (overall 2 doses at 6 and 30 h p.i.). MIC values of compound 7 and azithromycin were  $\leq 0.015$  and  $0.03 \mu\text{g/ml}$ , respectively. (a) CFU count per gram of lung tissue. Statistically significant reduction in CFU is designated by asterisk (\*= $p < 0.005$ ; \*\*= $p < 0.001$ ). (b) Concentration of compound in  $\mu\text{g/g}$  of lung tissue 14 h after the last dose, given as mean values  $\pm$ SEM.

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