Chloramphenicol and Metronidazole Derivatives of Azithromycin Overcome the Inducible Resistance to Macrolide Antibiotics

ECA 2023

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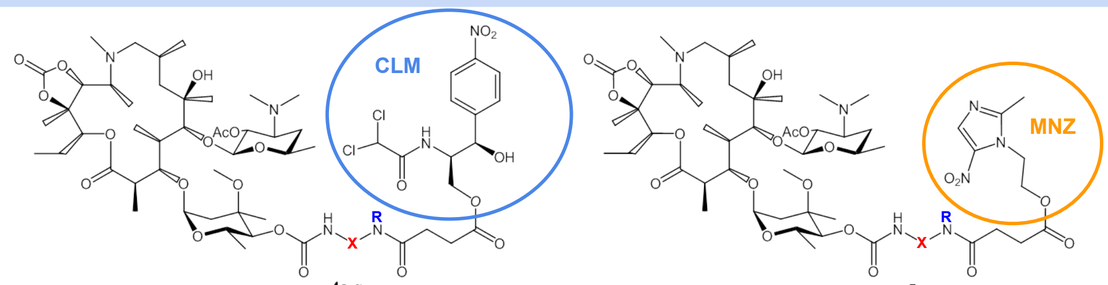
Introduction

The emergence and rapid development of microbial resistance to antibacterial drugs is one of the major problems for modern science and medicine. One of the methods being developed to address the problem is the design of hybrid antibacterial substances based on two different pharmacophores covalently linked to each other. In this work, we synthesized and characterized **two sets of hybrid compounds,** in which **azithromycin (AZT)** at the 4"-position **was bound to either chloramphenicol (CLM) or metronidazole (MNZ)** using linker fragments of different length and structure.



AZT-CLM derivatives							
Cmpd	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**
R	H	H	H	H	H	H	$(CH_2)_2OH$
X	$(CH_2)_2$	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_5$	$(CH_2)_7$	$((CH_2)_2O)_2(CH_2)_2$	$(CH_2)_2$
Yield, %	20	21	24	25	25	22	19
AZT-MZN derivatives							
Cmpd	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**
R	H	H	H	H	H	H	$(CH_2)_2OH$
X	$(CH_2)_2$	$(CH_2)_3$	$(CH_2)_4$	$(CH_2)_5$	$(CH_2)_7$	$((CH_2)_2O)_2(CH_2)_2$	$(CH_2)_2$
Yield, %	24	26	25	27	26	23	21

Methods & Results

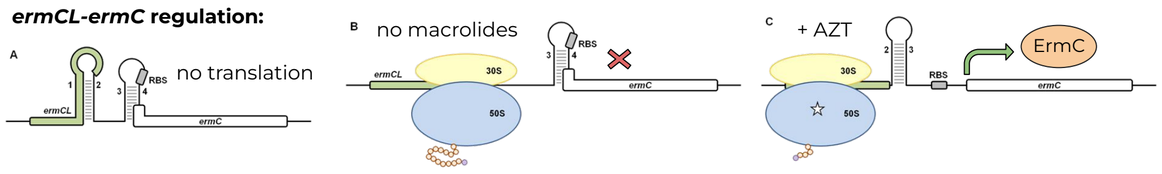
1. All AZT-derivatives do not inhibit the growth of *E. coli ΔtolC* pErmC bacteria constitutively resistant to macrolides. However, they were active against the *E. coli ΔtolC* pErmCL-ErmC strain inducibly resistant to macrolide antibiotics due to the ***ermCL*-dependent regulation of ErmC methyltransferase synthesis**.
E. coli ΔtolC and *E. coli ΔtolC* pERMZα were used as macrolide-sensitive controls.

Antibacterial activity (MIC, μg/mL) of AZT-derivatives

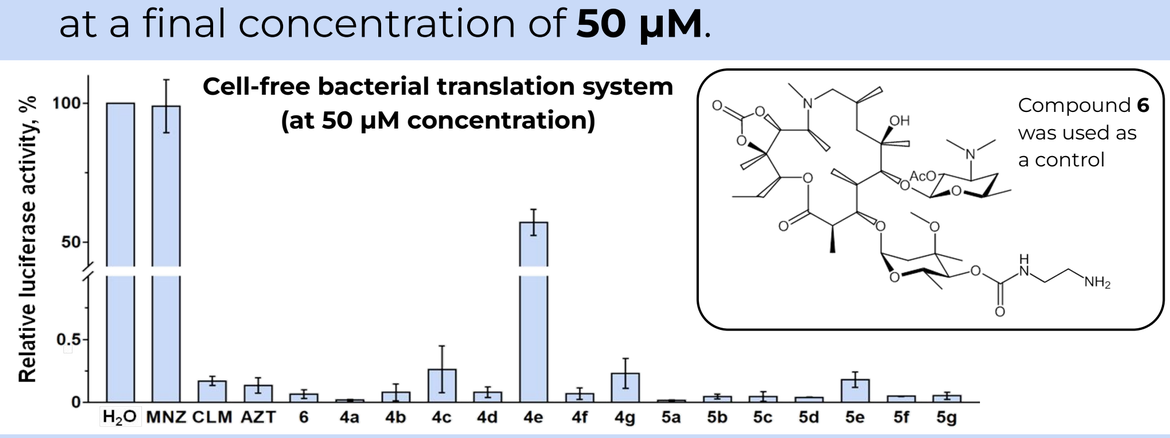
Strain	MNZ	CLM	AZT	ERY	4a	4b	4c	4d	4e	4f	4g	5a	5b	5c	5d	5e	5f	5g
E. coli ΔtolC	>200	1.6	0.4	3.1	12.5	12.5	12.5	12.5	>200	12.5	50	12.5	6.3	6.3	6.3	6.3	12.5	25
E. coli ΔtolC pERMZα	>200	1.6	0.8	1.6	12.5	12.5	12.5	12.5	>200	12.5	50	12.5	6.3	6.3	6.3	12.5	12.5	25
E. coli ΔtolC pErmCL-ErmC	>200	1.6	3.1	50	12.5	25	12.5	25	>200	25	100	12.5	6.3	12.5	12.5	12.5	12.5	25
E. coli ΔtolC pErmC	>200	1.6	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
Ratio*	-	**1:1**	**1:8**	**1:16**	**1:1**	**1:2**	**1:1**	**1:2**	-	**1:2**	**1:2**	**1:1**	**1:1**	**1:2**	**1:2**	**1:2**	**1:1**	**1:1**

* The ratio between MIC values for *E. coli ΔtolC* and *E. coli ΔtolC* pErmCL-ErmC strains

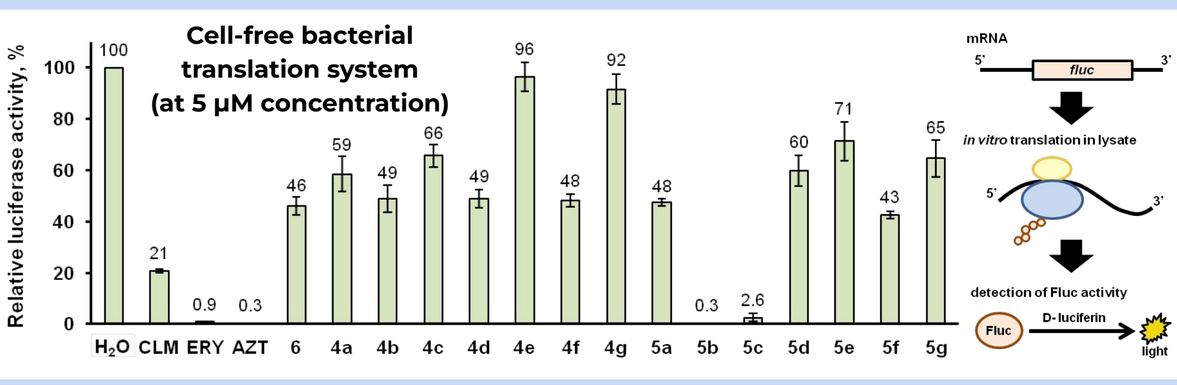
***ermCL-ermC* regulation:**



2. All AZT-derivatives except **4e** inhibit protein synthesis *in vitro* by 100% in a cell-free bacterial translation system at a final concentration of **50 μM**.



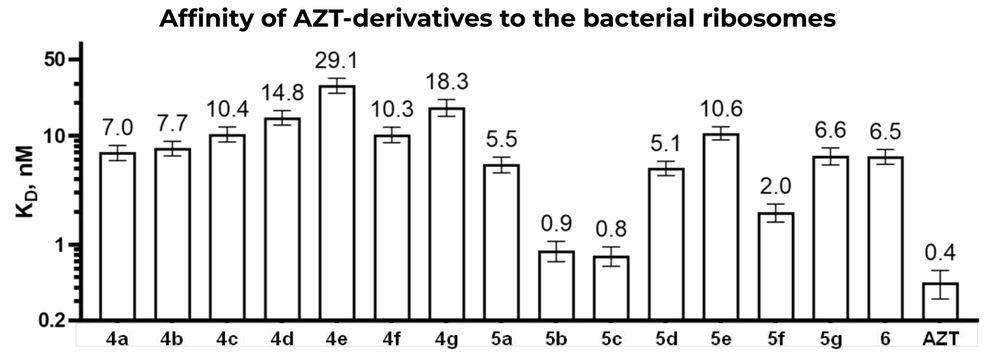
At a final concentration of **5 μM**, we could observe the difference in compounds effectiveness.



This research was funded by the Russian Science Foundation according to the research project No. 21-64-00006.

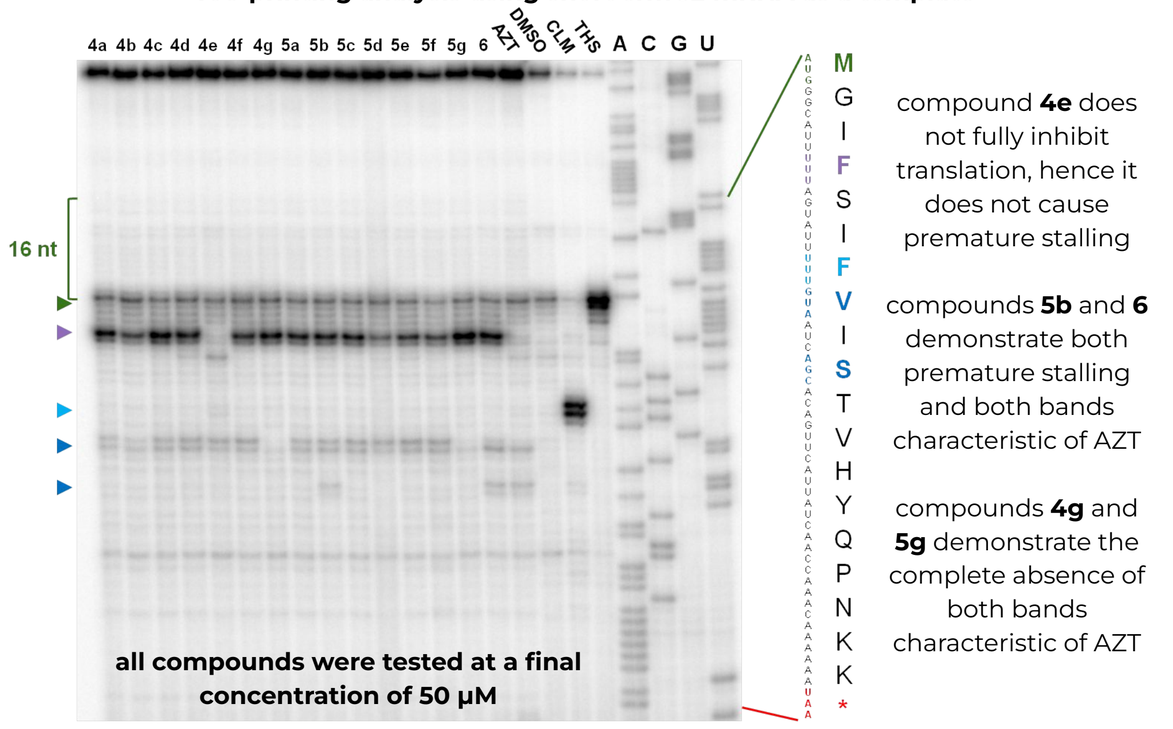
3. The activity of AZT-derivatives both *in vitro* and *in vivo* correlates well with apparent dissociation constants ($\mathbf{K_D}$) indicating their affinity to the 70S *E. coli* ribosomes (r = 0.8269). In general, compounds **5a-g** (especially **5b** and **5c**) are more active than **4a-g**. Compounds with linkers **e** and **g** seem to be less efficient.

Affinity of AZT-derivatives to the bacterial ribosomes



4. Toe-printing analysis in the presence of AZT-derivatives, using short *ermCL* mRNA as a template, revealed a **premature ribosome stalling**, as well as **the absence of ribosome arrest at positions characteristic of AZT** (and crucial for the regulation or ErmC synthesis).

Toe-printing analysis using short *ermCL* mRNA as a template



compound **4e** does not fully inhibit translation, hence it does not cause premature stalling

compounds **5b** and **6** demonstrate both premature stalling and both bands characteristic of AZT

compounds **4g** and **5g** demonstrate the complete absence of both bands characteristic of AZT

Conclusion

Novel derivatives of AZT have a preference to cause premature ribosome stalling during translation, which makes them active against bacterial strains inducibly resistant to the typical macrolide antibiotics.