The study of the correlation of the binding energy of peptide ligand complexes with hybrid antibiotics vancomycin-azithromycin and eremomycin-azithromycin with antibacterial activity

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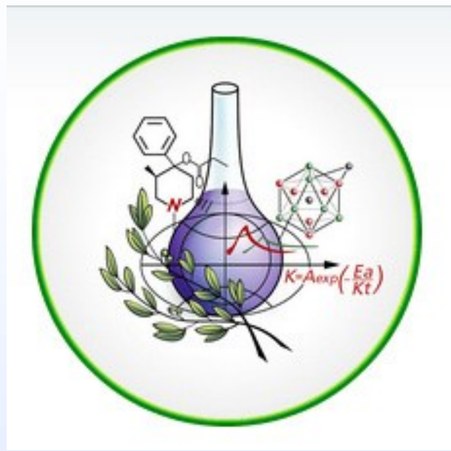
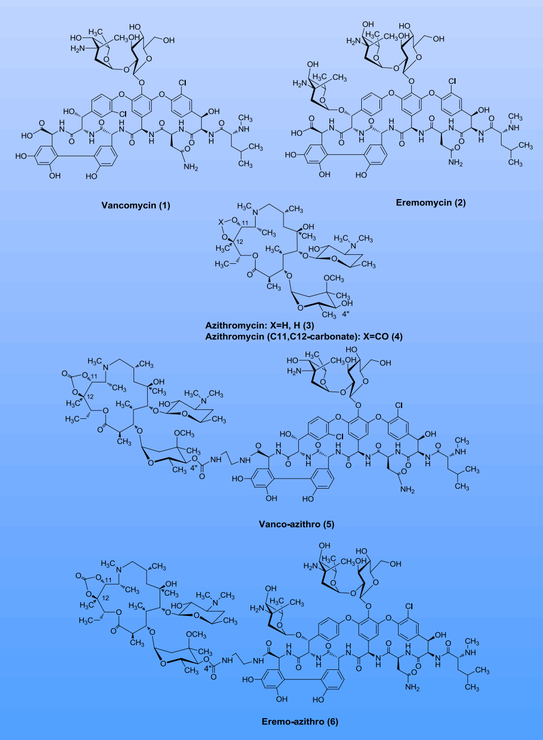


The natural glycopeptide antibiotics vancomycin **1** and eremomycin **2** (Scheme 1) are highly active against a wide range of Gram-positive *Staphylococcus aureus bacteria*, including methylclyline-resistant (MRSA), and *Enterococcus spp., Clostridium difficile*, etc., sensitive and resistant to betalactams, fluoroquinolones and tetracyclines. The mechanism of action of glycopeptide antibiotics is based on the inhibition of the synthesis of the bacterial cell wall by their strong binding to the N-acyl-D-Ala-D-Ala fragment of the growing peptidoglycan [1]. Five hydrogen bonds between the atoms of the target peptide and the "binding pocket" ensure a strong antibiotic-retaining of the target of the bacterial cell [2].



Scheme. 1 The structures of natural and hybrid antibiotics studied

Among broad-spectrum antibacterial agents, macrolide antibiotics that are active against many Gram-positive and Gram-negative bacteria are very effective. Among the antibiotics of this class, the semi-synthetic 15-member macrolide azithromycin **3** (Scheme 1) has the best pharmacological characteristics [3, 4]. The mechanism of action of macrolides is based on inhibition of protein synthesis, their target is the peptidyl-transferase center on the ribosome 50s.

However, even such highly effective reserve antibiotic as vancomycin and widely used antibiotic azithromycin become powerless over time in the fight against some strains of bacteria that have become resistant to them as a result of the mutation.

Resistance of bacteria to antibiotics is one of the most urgent problems of modern antibiotic therapy , which requires researchers to create new antibacterial agents that overcome this resistance . Highly effective antibacterial drugs of the new generation can be created by directional chemical modification of the most active natural antibiotics [10].

In connection with the foregoing, in recent years more and more attention of researchers has been attracted to hybrid structures in which molecules of antibiotics of different classes are connected by a covalent bond [3, 4]. Such drugs are promising for the treatment of infectious diseases caused by multiresistant bacteria. From the literature sources it is known that the properties of hybrid structures are not additive, i.e. the result of a simple addition of the properties of their constituent antibiotics. Covalently bound antibiotics may have new properties and have a wider spectrum of action than either of the components of the hybrid structure or when combined with their use. The study of the mechanisms of action of such hybrid antibiotics is especially important.

The present work is devoted to the study of antibacterial activity of hybrid antibiotic vancomycin-azithromycin- (C11, C12-carbonate) (5) and eremomycin-azithromycin- (C11, C12-carbonate) (6), and quantum-chemical calculations of the energy of their interaction with the peptide ligand - the model tripeptide α, ε-di-Ac-L-Lys-D-Ala-D-Ala by the semiempirical method PM6[7]. All quantum-chemical calculations of the interaction energy of antibiotics with the peptide ligand, the model tripeptide α, ε-di-Ac-L-Lys-D-Ala-D-Ala, were carried out using the standard software package Spartan-10 [8] by the semiempirical PM6 method [9].

The results of the study of the antibacterial activity of the hybrid antibiotics vancomycin-azithromycin (C11, C12-carbonate) (5) and eremomycin-azithromycin- (C11, C12-carbonate) (6) in comparison with the initial 1, 2 and azithromycin (3) are presented in Table 1 .

Table 1. The Study of antibacterial activity of antibiotics (1 - 3) and hybrid analogs (5, 6).

Штамм	МПК, μМ*				
	1	**2**	**3**	**5**	**6**
	Gram-positive				
I *Staphylococcus aureus* 25923 ATCC	0.7	0.15	1.2	**0.4** ↑	*0.8* ↓
II Staphylococcus epidermidis 533	0.3 ≈	0.15	2.5	0.4 ≈	*0.8* ↓
III Staphylococcus aureus 3797 (GISA)	2.7	2.4	>39.0	**1.7** ↑	**1.6** ↑
IV Staphylococcus aureus 1025 (GISA)	5.4	9.6	19.7	>13.4	**3.2** ↑
*V Enterococcus faecium*568	0.7	0.15	9.8	**0.4** ↑	*0.8* ↓
*VI Enterococcus faecium*569 (GRE)	>21.4	>19.2	9.8	>13.4	**3.2** ↑↑
VII Enterococcus faecalis 560 (GRE)	>21.4	>19.2	>39.0	13.4	**6.5** ↑
VIII Streptococcus pneumoniae 49619 ATCC (S)	1.3	0.6 ≈	9.8	**0.4** ↑	0.8 ≈
IX Streptococcus agalactis 52	5.46	0.6	>39.0	13.4	*3.2* ↓
	Gram- negative				
X E.coli 25922 ATCC	>21.4	>19.2	9.8	>13.4	>13.0

* Increase in activity (MPC, μM) of analogues 5 or 6 as a result of attachment to the antibiotic 1 or 2 of the azithromycin analogue (4) is marked with ↑ and in bold font, with two ↑↑ symbols indicating a significant, principal increase in activity. Reduction of the activity of analogues 5 or 6 as a result of attachment to the antibiotic 1 or 2 of the azithromycin analogue (4) is marked with ↓ and italics. The absence of the effect of the attached 4 in the hybrid analogs 5 and 6 on the activity of the initial 1 and 2 is indicated by the symbol ≈.

The results of quantum chemical calculations by the PM6 method have shown that hybrid analogs **5** and **6** are also able to bind to the model ligand α, ε-di-Ac-L-Lys-D-Ala-D-Ala ligand, like the original glycopeptides **1** And **2** (Scheme 1) (Tables 2 and 3). Figures 3 through 6 show the Stuart-Brigleb 3D models of the complexes of these antibiotics 1a, 2a and their hybrid analogs 5a and 6a with the model ligand.

Table 2. Binding energy of antibiotics 1, 2 and 5, 6 with ligand α, ε-di-Ac-L-Lys-D-Ala-D-Ala (LAA-) to form complexes 1a, 2a and 5a, 6a (methode PM6); The N-terminal group of the peptide bark is protonated ($NHCH3^+$).

Энергия связывания	Ванко (**1а**) ($1^+ + LAA^-$)	Эремо (**2а**) ($2^+ + LAA^-$)	Ванко-азитро (**5а**) ($5^+ + LAA^-$)	Эремо-азитро (**6а**) ($6^+ + LAA^-$)
ΔG_{298},* ккал/моль	-108.6	-97.7	-122.5	-101.5

The value of ΔG_{298} obtained by the method of B3LYP / 6-31G is -122.7 kcal / mol [21].

Table 3. Binding energy of antibiotics 1, 2 and 5, 6 with ligand α, ε-di-Ac-L-Lys-D-Ala-D-Ala (LAA-) to form complexes 1a, 2a and 5a, 6a (methode PM6), the N-terminal group of the peptide core is not protonated (NHCH3).

Энергия связывания	Ванко (**1б**) ($1 + LAA^-$)	Эремо (**2б**): ($2 + LAA^-$)	Ванко-азитро (**5б**) ($5 + LAA^-$)	Эремо-Азитро (**6б**) ($6 + LAA^-$)
ΔG_{298},* ккал/моль	-45.0	-61.0	-51.09	-42.9

* The value of ΔG_{298} obtained by the method of B3LYP / 6-31G* is -87.7 kcal / mol [22].

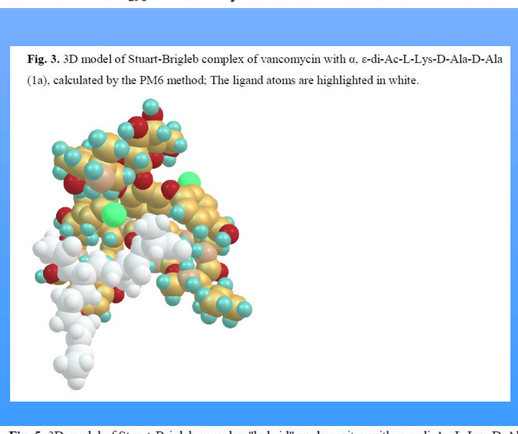


Fig. 3. 3D model of Stuart-Brigleb complex of vancomycin with α, ε-di-Ac-L-Lys-D-Ala-D-Ala (1a), calculated by the PM6 method; The ligand atoms are highlighted in white.

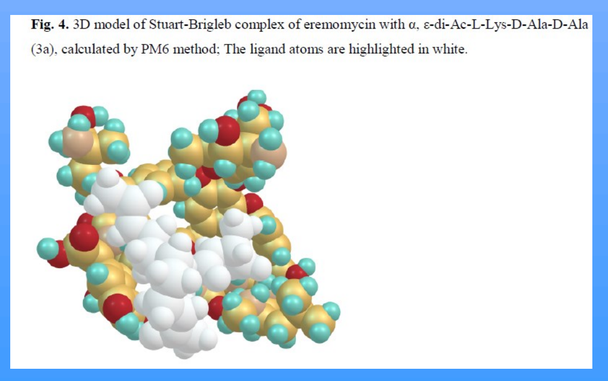


Fig. 4. 3D model of Stuart-Brigleb complex of eremomycin with α, ε-di-Ac-L-Lys-D-Ala-D-Ala (3a), calculated by PM6 method; The ligand atoms are highlighted in white.

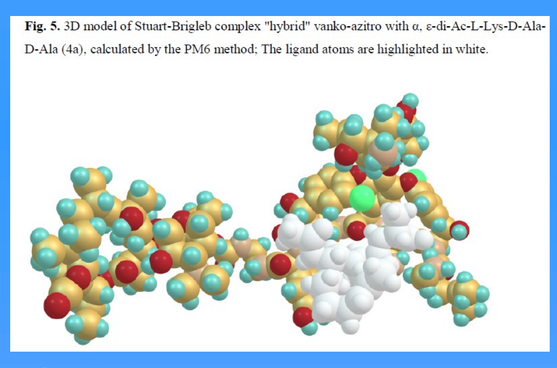


Fig. 5. 3D model of Stuart-Brigleb complex "hybrid" vanko-azitro with α, ε-di-Ac-L-Lys-D-Ala-D-Ala (4a), calculated by the PM6 method; The ligand atoms are highlighted in white.

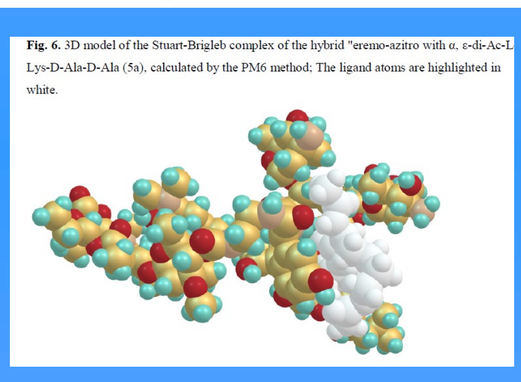


Fig. 6. 3D model of the Stuart-Brigleb complex of the hybrid "eremo-azitro with α, ε-di-Ac-L-Lys-D-Ala-D-Ala (5a), calculated by the PM6 method; The ligand atoms are highlighted in white.

Conclusion

1. The addition of the azithromycin derivative 4 molecule to vancomycin (1) or eremomycin (2) does not lead to loss of antibacterial activity against gram-positive bacteria (I, II, III, V and VIII), and quantum chemical calculations confirm that their activity is determined by Affinity to the target-D-Ala-D-Ala.

2. The higher activity of the ℏybrid"of vancomycin with azithromycin 5 compared with the activity of the ℏybrid"of eremomycin with azithromycin 6 against sensitive strains of gram-positive bacteria (I, II, III, V and VIII) correlates with the binding energies of ΔG_{298} of hybrid analogs with Model peptide α, ε-di-Ac-L-Lys-D-Ala-D-Ala, obtained by quantum chemical calculations for the non-protonated form of the antibiotic molecule.

3. In contrast to the hybrid analogue of vancomycin (5), the hybrid analog of eremomycin (6) shows marked activity against resistant Staphylococcus strains (GISA) (III, IV) and against resistant enterococci strains VI, VII (GRE type), which can be Explained by the effect of the azithromycin residue attached to the C-terminal group of the antibiotic 2 peptide core.

References

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