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MOLECULAR DOCKING OF 5-PHENYL-5,6-DIHYDROTETRAZOLO-[1,5-*c*]QUINAZOLINES TO RIBOSOMAL 50S PROTEIN L2P (2QEX)

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Summary. *Given the presence of antimicrobial activity for various tetrazole derivatives and its condensed analogues, it was proposed to analyze 13 novel 5-phenyl-5,6-dihydrotetrazolo[1,5-c]quinazolines for molecular docking to ribosomal 50S protein L2P (2QEX) in comparison with Tedizolid, tetrazole-oxazolidinone antibiotic. Hence, 4-(5-methyl-5,6-dihydrotetrazolo[1,5-c]-quinazolin-5-yl)benzoic acid* ***12*** *proved to be the most probable in terms of antimicrobial activity in vitro as a result of in silico molecular docking to antimicrobial 2QEX target.*

Keywords: *5-phenyl-5,6-dihydrotetrazolo[1,5-c]quinazolines, molecular docking, ribosomal 50S protein L2P (2QEX).*

Introduction. It's known, that structure-based drug design is usually used for binding energy analysis, interaction of ligand-protein, and evaluation of the conformational changes during the process of docking [1-4]. It predicts the preferred orientation of one molecule to a second when a ligand and a target are bound to each other to form a stable complex. So, one of the first steps to optimize the creation of new biologically active compounds among the selected structural series

of derivatives is to find a molecule with a proven mechanism of antimicrobial action to select a biological target for molecular docking.

The review by Gao F. *at al*. [5] covers the recent advances of tetrazole hybrids including tetrazole-azole, tetrazole-chalcone/coumarin/flavonoid, tetrazole-furan/pyrrole/thiophene, tetrazole-pyridine/pyrimidine, tetrazole-quinoline/quinolone, tetrazole-*β*-lactam and tetrazole-sugar hybrids as potential antibacterial agents. And Tedizolid 25 [5,6] (formally known as torezolid and TR-700, trade name Sivextro, Fig. 1) and Tedizolid phosphate - tetrazole-oxazolidinone antibiotics, have already been marketed in 2014 year for the treatment of acute bacterial skin and skin structure infections caused by certain susceptible bacteria, including *S. aureus* (also methicillin-susceptible *S. aureus*/MSSA, methicillin-resistant *S. aureus*/MRSA, linezolid-resistant staphylococci), various Streptococcus species (*S. pyogenes, S. agalactiae*, *S. anginosus*, *S. intermedius* and *S. constellatus),* and *E. faecalis.*

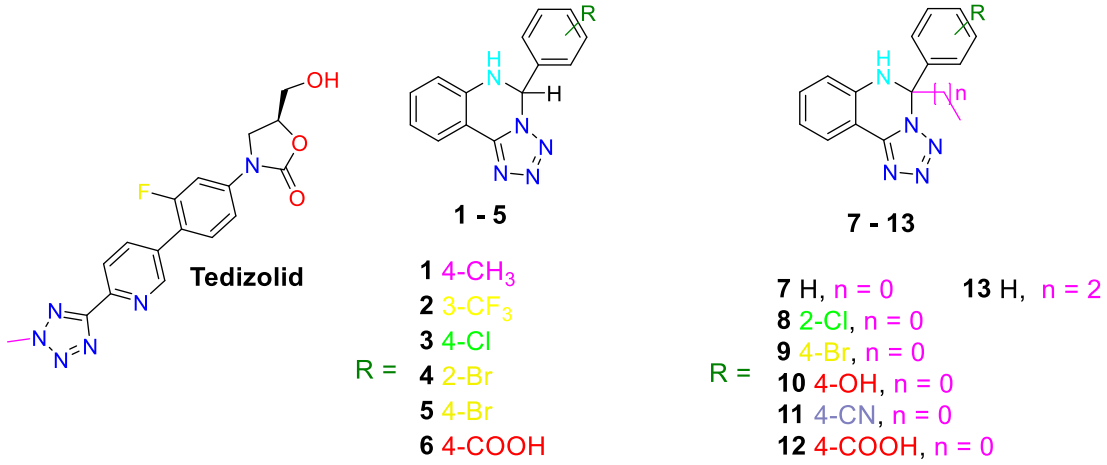

Fig. 1. Structural formula of Tedizolid as antimicrobial and structural analogue and proposed 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]quinazolines

It was shown that Teridozolid can exert bacteriostatic activity *via* inhibition of protein synthesis by binding to the 50S ribosomal subunit of the bacteria [7]. Protein synthesis involves the action of ribosomes, multi-subunit complexes composed of both protein and ribosomal RNA (rRNA) substituents. The bacterial 70S ribosome comprises a small (30S) and a large (50S) subunit. Translocation along the length of a messenger RNA and concomitant protein synthesis involves the action of the A, P, and E sites of the peptidyltransferase center, which accepts charged aminoacyl-tRNAs and catalyzes the formation of peptide bonds between them.

Nevertheless, sequence analysis of 23S rRNA, *rrlC* gene and ribosomal protein genes *rplA* and *rspQ,* performed on the recovered WIS 423 tedizolid-mutant strain, revealed that this strain possessed the G2576T mutation in one of the six copies of 23S rRNA. These results support the evidence that spontaneous mutation leading to reduced Tedizolid susceptibility is present at low frequency events, including for CF-*S. aureus* strains [8].

Aim. Thus, based on the abovementioned data, new 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]quinazolines could be promising antimicrobials, and to predict their activity by *in silico* molecular docking, 50S ribosomal protein was selected as the antimicrobial target.

Materials and Method. Macromolecule from Protein Data Bank (PDB) was used as a biological target, namely 50S ribosomal protein L2P (PDB ID - 2QEX) [9]. Tedizalid [7] was chosen as a reference. The 14 mol files of 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]quinazoline derivatives and Tedizalid were drawn by ChemDraw Professional 15.0 and optimized by HyperChem 8.0.8; mol files were converted to pdb by Open Babel GUI 2.3.2; pdb files were converted to pdbqt by AutoDocTools 1.5.6. Vina 1.1.2 was used to carry out docking studies [10]. Discovery Studio v17.2.0.16349 was used for visualization.

Results and discussion. The studied 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]-quinazolines differ in hydrogen-methyl-propyl substituent in the 5th position, or/and have thrifluoromethyl, halogen, hydroxyl, cyano or carboxylic group in the phenyl substituent (Fig. 1). The choice of substituent was conditioned by the fact, that incorporation of fluorine to phenyl ring can improve the therapeutic efficacy due to hydrogen bonding interactions at the active sites of enzyme [11], as well as carboxylic and hydroxyl groups enhance antibacterial properties [12].

In the result of molecular docking, the following affinities were found (Table 1).

Table 1

Affinity to binding sites of 2QEX, with decreasing of score

#	12	Tedizolid	11	6	10	2	9
kcal/mol	-8.7	-8.4	-8.2	-8.1	-8.0	-8.0	-7.9
#	8	7	5	1	3	4	13
kcal/mol	-7.5	-7.4	-7.3	-7.3	-7.2	-7.2	-7.1

As it can be seen from Table 1, the best affinity was shown by substance **12**, scoring even higher (-8.7 kcal/mol), than the reference Tedizolid (-8.4 kcal/mol).

All other tetrazole derivatives had affinity lower than the reference compound. Still substance **11**, **6**, **10**, **2** and **9** had affinity score more than 8 kcal/mol.

Analyzing the structure-affinity correlation, a score of **6** was of lower value than that of **12**, and the same for: **5** / **9**, and **13** / 7. So, introduction of carboxylic group to the 4th position of 5-phenyl substituent along with addition of 5-methyl substituent into 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]quinazoline were the most favorable suggestions for substance to be promising ligand for the studied target, than prolongation of alkyl substituent to propyl into 5th position. Also, 4-cyano or 4-hydroxy group into the 5-phenyl ring were preferable groups too.

In the results of data comparisons of **5** to **4**, **5** to **3**, it was found that position and type of halogen atoms were practically not important. However, bromide substituent in the 4th position compared to its 2nd position in the 5-phenyl ring increased the affinity score to 0.1. Also, as it was expected, the introduction of 3-trifluorophenyl substituent of **2,** than of 4-methylphenyl of **1** or 4-chlorophenyl of **3** was more suitable for the studied target.

According to the number of the formed bonds (Table 2), substance **2** made the highest number of them - 10 bonds due to CF_3 group. Among them were Carbon and Pi-Donor Hydrogen Bonds, electrostatic Pi-Anion bonds and Halogen (Fluorine) ones. And Tedizolid had formed only 5 bonds of the same type, except Halogen one.

Among all substances, the shortest bonds were Conventional Hydrogen bonds of 2.17-2.81Å of **11**, **10**, **3**, **5**, **1**, **6**, Tedizolid, and **13**. Also 3.12-3.43Å Carbon or Conventional Hydrogen Bonds or Halogen one were found for **2**, **12**, and **5**.

Table 2

Descriptives of formed bonds to 50S ribosomal protein L2P (2QEX)

#	Bond from - to	Distance, Å	Category	Type
Tedizolid	:UNL1:H - 0:G518:O6	2.69	HB*	Conventional HB
	0:U510:C4' - :UNL1:O	3.62	HB	Carbon HB
	:UNL1:C - 0:G518:N7	3.66	HB	Carbon HB
	0:G518:OP1 - :UNL1	4.97	Electrostatic	Pi-Anion
	0:G518:OP2 - :UNL1	3.85	Electrostatic	Pi-Anion
1	:UNL1:H - 0:G518:O2'	2.43	HB	Conventional HB
	:UNL1:C - 0:G23:O6	3.57	HB	Carbon HB
	0:G21:OP2 - :UNL1	3.95	Electrostatic	Pi-Anion
	0:G21:OP2 - :UNL1	3.36	Electrostatic	Pi-Anion
	0:G20 - :UNL1:C	4.40	Hydrophobic	Pi-Alkyl
2	0:G21:C5' - :UNL1:F	3.12	HB	Carbon HB
	0:G21:C5' - :UNL1:F	3.18	HB	Carbon HB
	0:G23:O6 - :UNL1:F	3.16	Halogen	Halogen (Fluorine)
	0:G23:O6 - :UNL1:F	3.51	Halogen	Halogen (Fluorine)
	0:G21:OP2 - :UNL1	4.44	Electrostatic	Pi-Anion
	0:G518:OP2 - :UNL1	3.95	Electrostatic	Pi-Anion
	0:G518:OP2 - :UNL1	3.87	Electrostatic	Pi-Anion
	0:A519:OP2 - :UNL1	4.85	Electrostatic	Pi-Anion
	0:G518:O2' - :UNL1	3.68	HB	Pi-Donor HB
	0:U1338:O2' - :UNL1	3.97	HB	Pi-Donor HB
3	:UNL1:H - 0:G21:OP1	2.35	HB	Conventional HB
	0:G21:OP2 - :UNL1	3.52	Electrostatic	Pi-Anion
	0:G21 - :UNL1:CL	5.30	Hydrophobic	Pi-Alkyl
4	0:G21:C5' - :UNL1:N	3.54	HB	Carbon HB
	0:G21:OP2 - :UNL1	3.45	Electrostatic	Pi-Anion
	0:G21 - :UNL1	5.69	Hydrophobic	Pi-Pi Stacked
5	:UNL1:H - 0:G518:O2'	2.42	HB	Conventional HB
	0:G21:C5' - :UNL1:N	3.43	HB	Carbon HB
	:UNL1:C - 0:G23:O6	3.60	HB	Carbon HB
	0:G21:OP1 - :UNL1	3.80	Electrostatic	Pi-Anion
	0:G21:OP2 - :UNL1	3.51	Electrostatic	Pi-Anion
	0:G20 - :UNL1:BR	4.72	Hydrophobic	Pi-Alkyl
6	:UNL1:H - 0:G23:O6	2.66	HB	Conventional HB
	:UNL1:H - 0:G518:O2'	2.81	HB	Conventional HB
	:UNL1:H - 0:A519:OP2	2.66	HB	Conventional HB
	0:G21:OP2 - :UNL1	3.43	Electrostatic	Pi-Anion
7	0:G21:OP2 - :UNL1	3.63	Electrostatic	Pi-Anion
	0:U517:OP1 - :UNL1	4.03	Electrostatic	Pi-Anion
	0:G518:O2' - :UNL1	3.91	HB	Pi-Donor HB
8	0:G21:OP2 - :UNL1	3.97	Electrostatic	Pi-Anion
	0:U517:OP2 - :UNL1	4.31	Electrostatic	Pi-Anion
	0:G518 - :UNL1	4.57	Hydrophobic	Pi-Pi T-shaped

Continuation of the table 2

#	Bond from - to	Distance, Å	Category	Type
9	0:G21:OP2 - :UNL1	3.56	Electrostatic	Pi-Anion
	0:U517:OP1 - :UNL1	4.05	Electrostatic	Pi-Anion
	0:G518:O2' - :UNL1	3.85	HB	Pi-Donor HB
10	:UNL1:H - 0:A516:OP1	2.59	HB	Conventional HB
	:UNL1:H - 0:G518:O6	2.20	HB	Conventional HB
	0:G518:C8 - :UNL1:N	3.58	HB	Carbon HB
	0:G21:OP2 - :UNL1	3.53	Electrostatic	Pi-Anion
	0:G518:O2' - :UNL1	3.72	HB	Pi-Donor HB
	0:G518 - :UNL1	4.94	Hydrophobic	Pi-Pi T-shaped
11	:UNL1:H - 0:G518:OP2	2.17	HB	Conventional HB
	0:G21:OP2 - :UNL1	4.63	Electrostatic	Pi-Anion
	0:G518:OP1 - :UNL1	3.82	Electrostatic	Pi-Anion
	0:A519:OP1 - :UNL1	4.57	Electrostatic	Pi-Anion
	0:G518:O2' - :UNL1	3.68	HB	Pi-Donor HB
	0:U1338:O2' - :UNL1	3.83	HB	Pi-Donor HB
	0:G518 - :UNL1	4.53	Hydrophobic	Pi-Pi T-shaped
	0:A1321 - :UNL1:C	4.84	Hydrophobic	Pi-Alkyl
	0:A1337 - :UNL1:C	4.88	Hydrophobic	Pi-Alkyl
12	0:C515:O2' - :UNL1:O	3.23	HB	Conventional HB
	0:C515:O2' - :UNL1:O	3.22	HB	Conventional HB
	0:A511:C4' - :UNL1:O	3.42	HB	Carbon HB
	0:G21:OP2 - :UNL1	3.49	Electrostatic	Pi-Anion
	0:U517:OP1 - :UNL1	3.92	Electrostatic	Pi-Anion
	0:G518:O2' - :UNL1	3.88	HB	Pi-Donor HB
13	:UNL1:H - 0:G24:O6	2.77	HB	Conventional HB
	0:G21:OP2 - :UNL1	3.84	Electrostatic	Pi-Anion

** HB –Hydrogen Bond.*

And, in Figure 2, the 2D pictures of substances with highest affinity (Tedizolid and 12) to 2QEX are shown.

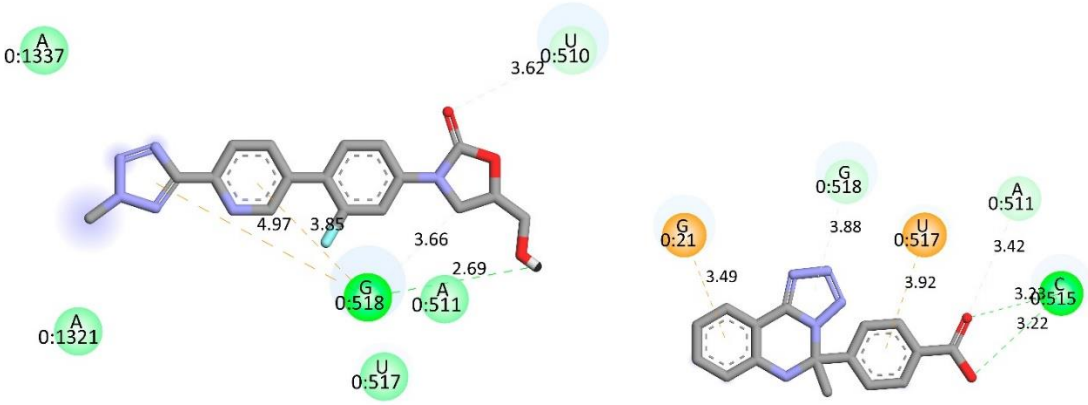

Fig. 2. Visual representation (2D) of the Tedizolid *versus* lead compound 12 showing bonds formation in the active site of 50S ribosomal protein L2P (2QEX). Pale green – van der Waals interaction or π -Donor Hydrogen bond, green – conventional Hydrogen Bond, orange – electrostatic π -anion

Thus, Tedizolid has formed three Hydrogen Bonds: one shortest Conventional with glutamine (G518, 2.69Å), and two Carbon with selenocysteine (U510 and U518). Additionally two electrostatic Pi-anion Bonds with glutamine (G518). While 12 has made four Hydrogen bonds: one Conventional with glutamine (G24), two shortest Conventional with cysteine (C515, of 3.22 and 3.23 Å), one Carbon with alanine (A511), and one Pi-Donor with glutamine (G518). Also, two electrostatic Pi-anion Bonds with glutamine (G21) and selenocysteine (U517).

Conclusions. The 4-(5-methyl-5,6-dihydrotetrazolo(1,5-*c*]quinazolin-5-yl)-benzoic acid 2.12 proved to be the most probable in terms of antimicrobial activity *in vitro* as a result of *in silico* molecular docking to the antimicrobial target 2QEX. Prediction of these series' ADME properties is ongoing too.

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References:

[1] Bohacek, R. S., McMartin, C., Guida, W. C. (1996). The art and practice of structure-based drug design: a molecular modeling perspective. *Med. Res. Rev*. 16(1), 3-50.
[2] Wilson, G. L., Lill, M. A. (2011). Integrating structure-based and ligand-based approaches for computational drug design. *JFmc,* 3(6), 735-750.
[3] Buchanan, S G. (2002). Structural genomics: bridging functional genomics and structure-based drug design. *Curr. Opin. Drug Discov. Dev*., 5(3), 367-381.
[4] Singh, S., Malik, B. K., Sharma, D. K. (2006). Molecular drug targets and structure based drug design. A holistic approach. *Bioinform.*, 1(8), 314-320.
[5] Gao, F., Xiao, J., Huang, G. (2019). Current scenario of tetrazole hybrids for antibacterial activity. *Eur. J. Med. Chem*., (184), 111744.
[6] Carena, A. A., Stryjewski, M.E. (2020). Tedizolid (torezolid) for the treatment of complicated skin and skin structure infections. *Expert Rev. Clin. Pharmacol*., 13(6), 577-592.
[7] *Drugbank online. Tedizolid phosphate*. (2022). Retrieved from: https://go.drugbank.com/drugs/DB09042.
[8] Roch, M., Varela, M. C., Taglialegnam, A., Rosato, A. E. (2020). Tedizolid is a promising antimicrobial option for the treatment of *Staphylococcus aureus* infections in cystic fibrosis patients. *J. Antimicrob. Chemother*., 75(1), 126-134.
[9] *Negamycin Binds to the Wall of the Nascent Chain Exit Tunnel of the 50S Ribosomal Subunit*. (2008). Retrieved from: https://www.rcsb.org/structure/2QEX
[10] Trott, O., Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comput. Chem.,* 31, 455-461.
[11] Anzahaee, M. Y., Watts, J. K., Alla, N. R., Nicholson, A. W., Damha, M. J. (2011). Energetically important C-H· · ·F-C pseudohydrogen bonding in water: evidence and application to rational design of oligonucleotides with high binding affinity. *J. Am. Chem. Soc*., 133, 728–731.
[12] Beek, A. T., Brul, S. (2010). To kill or not to kill Bacilli: Opportunities for food biotechnology. *Cur. Opin. Biotechnol*., 21, 168-174.

