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MOLECULAR DOCKING OF 5-PHENYL-5,6-DIHYDROTETRAZOLO-[1,5-*c*]QUINAZOLINES TO PENICILLIN-BINDING PROTEIN 2X (PBP 2X) AND PRELIMINARY RESULTS OF ANTIFUNGAL ACTIVITY

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Summary. *Affinity of thirteen 5-phenyl-5,6-dihydrotetrazolo[1,5-c]quinazolines and reference Tedizolid is calculated to penicillin-binding protein 2X (PBP 2X) (PDB ID: 2ZC4). The lead-compounds are proposed based on presented results, previous affinity towards ribosomal 50S protein L2P (2QEX), and their ADME profile. Besides, substance* **12** *has already shown good preliminary antifungal results towards C. albicans.*

Keywords: *5-phenyl-5,6-dihydrotetrazolo[1,5-c]quinazolines, molecular docking, penicillin-binding protein 2X (PBP 2X), antifungal activity, C. Albicans*

Introduction. As a result of the previously reported [1] *in silico* molecular docking to the antimicrobial target ribosomal 50S protein L2P (PDB ID: 2QEX, 4-(5-methyl-5,6-dihydrotetrazolo(1,5-*c*]quinazolin-5-yl)benzoic acid **12** (Fig. 1) proved to be the most probable in terms of *in vitro* antimicrobial activity.

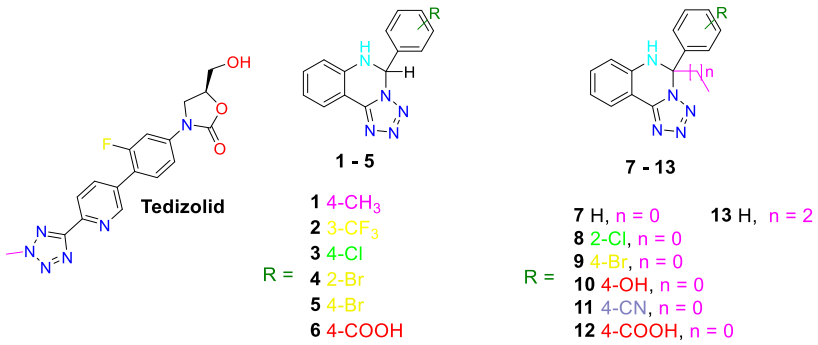

Fig. 1. Investigated 5-phenyl-5,6-dihydrotetrazolo[1,5-c]quinazolines, and Tedizolid.

Besides, prediction of these series' ADME properties [2], demonstrated that 4-(5-methyl-5,6-dihydrotetrazolo[1,5-*c*]quinazolin-5-yl)phenol (**10**) was the most promising molecule for synthesis and drug purposeful search, along with 4-(5,6-dihydrotetrazolo[1,5-*c*]quinazolin-5-yl)benzoic acid (**6**), and its 5-methyl analogue **12**, although the two latter permeate the BBB.

Aim: Based on the above-mentioned data, to confirm the affinity of 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]quinazolines towards antimicrobial targets, additional *in silico* molecular docking was proposed to carry out towards penicillin-binding protein 2X (PBP 2X) from *Streptococcus pneumonia*.

Materials and methods. *Molecular docking.* Macromolecule from Protein Data Bank (PDB) was used as a biological target, namely penicillin-binding protein 2X (PBP 2X) from *Streptococcus pneumonia* (PDB ID: 2ZC4) [3]. Tedizalid [4] was chosen as a reference. The 14 mol files of 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]-quinazoline derivatives with Tedizalid were drawn by MarvinSketch 20.20.0 and saved in mol format; 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 [5]. The following grid box was used: fcenter_x = 34.589, center_y = 61.828, center_z = -30.442, size_x = 18, = 18 size_y, size_z = 18. Discovery Studio v17.2.0.16349 was used for visualization.

Results and discussion. The following affinity scores towards penicillin binding protein 2X were obtained (Table 1).

Table 1

Affinity of substances to binding sites of penicillin binding protein 2X

#	2	11	1	12	5	9	6
kcal/mol	-8.9	-8.6	-8.6	-8.6	-8.5	-8.4	-8.4
#	Tedizolid	3	10	13	7	8	4
kcal/mol	-8.4	-8.3	-8.1	-8.0	-8.0	-8.0	-7.9

As it can be seen, substance **2** from the 6th place of previous affinity towards ribosomal 50S protein L2P [1] moved to the first one, while the **12**, **11** and **1** are the second place with -8.6 kcal/mol. Moreover, almost half of the substances had affinity to 2ZC4 better than the Tedizolid scoring higher than -8.4 kcal/mol.

Comparing chemical structure of substances, the following correlations were obtained. So, the highest affinity was shown by 5-(3-(trifluoromethyl)phenyl)-5,6-dihydrotetrazolo[1,5-*c*]quinazoline **2** (-8.9 kcal/mol) (Fig. 1). Interesting, that influence of 4-CN (**11**) and 4-COOH (**12**) in the phenyl ring at the 5th position of dihydrotetrazolo[1,5-*c*]quinazoline along with methyl group in the same place, and

4-CH_3 substituent in the phenyl ring for **1** had the same impact for affinity towards 2ZC4 (-8.6 kcal/mol). Interesting, that the prolongation of the alkyl substituent in the 5th position of **7** *vs* **13** itself has no effect. When bearing 5-(4-bromophenyl) substituent of **5** and **9**, absence of 5-methyl for **5** is favorable at 0.1 kcal/mol. And, when proposing 4-(5-methyl-5,6-dihydrotetrazolo[1,5-*c*]quinazolin-5-yl)benzoic acid **12**, it has better affinity in 0.2 kcal/mol than acid **6** without 5-metyl substituent. Also, for **6** (4-COOH, no 5-CH_3) and **9** (4-Br, 5-CH_3) there was no difference in affinity (-8.4 kcal/mol). Notable, that 4th position of bromo substituent in the 5-phenyl ring (**5**) is preferable than 2nd (**4**, in 0.6 kcal/mol). Change of 4-chloro substituent of **3** to 4-bromo substituent of **5** increased its affinity to 0.2 kcal/mol.

Moreover, there were formed more bonds to penicillin-binding protein 2X than towards ribosomal 50S protein L2Pm [1] (Table 2).

Table 2

Descriptives of formed bonds to penicillin binding protein 2X

#	Bond from - to	Dist., Å	Category	Type
Tedizolid	E:SER337:OG - :UNL1:F	3.44	HB; Halogen	Conventional HB; Halogen (Fluorine)
	E:ASN397:ND2 - :UNL1:N	2.90	HB	Conventional HB
	E:THR550:N - :UNL1:F	3.06	HB; Halogen	Conventional HB; Halogen (Fluorine)
	E:THR550:OG1 - :UNL1:O	3.04	HB	Conventional HB
	:UNL1:H - E:THR526:O	1.97	HB	Conventional HB
	:UNL1:C - E:THR526:O	3.45	HB	Carbon HB
	:UNL1:C - E:SER548:OG	3.62	HB	Carbon HB
	E:THR550:OG1 - :UNL1	3.66	HB	Pi-Donor HB
1	E:LYS340:NZ - :UNL1:N	3.30	HB	Conventional HB
	E:SER395:OG - :UNL1:N	2.93	HB	Conventional HB
	:UNL1:C - E:TRP374	3.57	Hydrophobic	Pi-Sigma
	E:TRP374 - :UNL1	3.86	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	4.48	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	5.16	Hydrophobic	Pi-Pi T-shaped
	E:TRP374 - :UNL1:C	4.38	Hydrophobic	Pi-Alkyl
2	E:SER337:OG - :UNL1:N	2.85	HB	Conventional HB
	E:TRP374:NE1 - :UNL1:F	3.03	HB; Halogen	Conventional HB; Halogen (Fluorine)
	E:ASN397:ND2 - :UNL1:N	3.05	HB	Conventional HB
	E:SER395:CB - :UNL1:F	3.68	HB	Carbon HB
	E:TRP374 - :UNL1	4.08	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	4.64	Hydrophobic	Pi-Pi Stacked
	E:ASP373:C,O;TRP374:N - :UNL1	4.21	Hydrophobic	Amide-Pi Stacked
	E:TRP374 - :UNL1:C	3.88	Hydrophobic	Pi-Alkyl
	E:TRP374 - :UNL1:C	4.28	Hydrophobic	Pi-Alkyl
3	E:LYS340:NZ - :UNL1:N	3.21	HB	Conventional HB
	E:SER395:OG - :UNL1:N	2.93	HB	Conventional HB
	:UNL1:CL - E:TRP374	3.59	Hydrophobic	Pi-Sigma
	E:TRP374 - :UNL1	3.94	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	4.47	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	5.19	Hydrophobic	Pi-Pi T-shaped
	E:TRP374 - :UNL1:CL	4.60	Hydrophobic	Pi-Alkyl
4	E:LYS340:NZ - :UNL1:N	3.17	HB	Conventional HB
	E:SER395:OG - :UNL1:N	2.93	HB	Conventional HB
	E:TRP374 - :UNL1	4.50	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	5.04	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1:BR	4.28	Hydrophobic	Pi-Alkyl
	E:TRP374 - :UNL1:BR	5.03	Hydrophobic	Pi-Alkyl
5	E:LYS340:NZ - :UNL1:N	3.26	HB	Conventional HB
	E:SER395:OG - :UNL1:N	2.97	HB	Conventional HB
	E:TRP374 - :UNL1	3.77	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	4.23	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	5.10	Hydrophobic	Pi-Pi T-shaped
	E:ASP373:C,O; TRP374:N - :UNL1	4.64	Hydrophobic	Amide-Pi Stacked
	E:TRP374 - :UNL1:BR	4.87	Hydrophobic	Pi-Alkyl
	E:TRP374 - :UNL1:BR	3.85	Hydrophobic	Pi-Alkyl

Continuation of Table 2

Descriptives of formed bonds to penicillin binding protein 2X

#	Bond from - to	Dist., Å	Category	Type
6	E:ASN397:ND2 - :UNL1:N	2.90	HB	Conventional HB
	:UNL1:H - E:THR550:O	2.72	HB	Conventional HB
	E:ASN397:ND2 - :UNL1	3.48	HB	Pi-Donor HB
	:UNL1:H - E:TRP374	2.76	HB	Pi-Donor HB
	E:TRP374 - :UNL1	3.62	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	4.53	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	4.76	Hydrophobic	Pi-Pi T-shaped
7	E:SER337:OG - :UNL1:N	3.07	HB	Conventional HB
	E:SER548:OG - :UNL1:N	2.80	HB	Conventional HB
	E:THR550:OG1 - :UNL1:N	3.10	HB	Conventional HB
	:UNL1:C - E:TRP374	3.99	Hydrophobic	Pi-Sigma
	E:TRP374 - :UNL1	4.91	Hydrophobic	Pi-Pi Stacked
8	E:SER337:OG - :UNL1:N	3.10	HB	Conventional HB
	E:SER548:OG - :UNL1:N	2.80	HB	Conventional HB
	E:THR550:OG1 - :UNL1:N	3.12	HB	Conventional HB
	:UNL1:C - E:TRP374	3.69	Hydrophobic	Pi-Sigma
	E:TRP374 - :UNL1	4.97	Hydrophobic	Pi-Pi Stacked
9	E:SER337:OG - :UNL1:N	2.98	HB	Conventional HB
	E:SER395:OG - :UNL1:N	2.88	HB	Conventional HB
	E:SER548:OG - :UNL1:N	2.83	HB	Conventional HB
	E:THR550:OG1 - :UNL1:N	2.99	HB	Conventional HB
	E:SER548:OG - :UNL1	3.66	HB	Pi-Donor HB
	E:THR550:OG1 - :UNL1	4.18	HB	Pi-Donor HB
	E:TRP374 - :UNL1	4.80	Hydrophobic	Pi-Pi Stacked
	:UNL1:BR - E:MET527	4.17	Hydrophobic	Alkyl
	:UNL1:BR - E:LEU600	5.08	Hydrophobic	Alkyl
10	E:SER337:OG - :UNL1:N	2.97	HB	Conventional HB
	E:SER395:OG - :UNL1:N	2.95	HB	Conventional HB
	E:SER548:OG - :UNL1:N	2.87	HB	Conventional HB
	E:THR550:OG1 - :UNL1:N	3.08	HB	Conventional HB
	E:SER548:OG - :UNL1	3.95	HB	Pi-Donor HB
	E:TRP374 - :UNL1	4.87	Hydrophobic	Pi-Pi Stacked
11	E:SER337:OG - :UNL1:N	2.90	HB	Conventional HB
	E:SER395:OG - :UNL1:N	2.83	HB	Conventional HB
	E:SER548:OG - :UNL1:N	2.96	HB	Conventional HB
	E:THR550:OG1 - :UNL1:N	2.96	HB	Conventional HB
	E:GLY549:CA - :UNL1:N	3.68	HB	Carbon HB
	E:SER548:OG - :UNL1	3.60	HB	Pi-Donor HB
	E:TRP374 - :UNL1	4.82	Hydrophobic	Pi-Pi Stacked
	:UNL1:C - E:MET527	4.28	Hydrophobic	Alkyl
	:UNL1:C - E:LEU600	4.46	Hydrophobic	Alkyl
12	E:GLY549:N - :UNL1:N	3.16	HB	Conventional HB
	E:THR550:OG1 - :UNL1:N	2.80	HB	Conventional HB
	:UNL1:H - E:ASN397:OD1	2.29	HB	Conventional HB
	E:SER548:OG - :UNL1	3.62	HB	Pi-Donor HB
	E:THR550:OG1 - :UNL1	3.24	HB	Pi-Donor HB
	E:TRP374 - :UNL1	4.61	Hydrophobic	Pi-Pi Stacked
	E:TRP374 - :UNL1	4.91	Hydrophobic	Pi-Pi T-shaped
13	E:SER337:OG - :UNL1:N	3.07	HB	Conventional HB
	E:LYS547:NZ - :UNL1:N	3.35	HB	Conventional HB
	E:SER548:OG - :UNL1:N	2.75	HB	Conventional HB
	E:SER548:OG - :UNL1	3.76	HB	Pi-Donor HB
	E:TRP374 - :UNL1	5.01	Hydrophobic	Pi-Pi Stacked

*HB - Hydrogen Bond

Thus, the 2, 9, 11 had the highest number of 9 bonds, among which were Conventional, Carbon and π-Donor Hydrogen bonds, hydrophobic π-π or Amid-π Stacked and Alkyl bonds. Afterwards went Tedizolid with 8 bonds, among which appeared Fluorine Hydrogen bond.

Interesting, that the shortest Conventional Hydrogen Bonds were formed by Tedizolid with a distance of 1.97 Å with THR526, and 12 - with 2.29 Å with ASN397.

Moreover, all substances formed Conventional Hydrogen Bonds of 2.72 – 3.35 Å, among those 2, 6-13 had the shortest ones. And, substance 6 had the shortest π-donor Hydrogen bond (2.76 Å), and 2 – the shortest Halogen one (3.03 Å).

Thus, the following Figure 2 shows a 2D representation of Tedizolid and substance 12 (with not the highest affinity, but still higher than the reference, and with good ADME results) of binding to the 2X penicillin-binding protein.

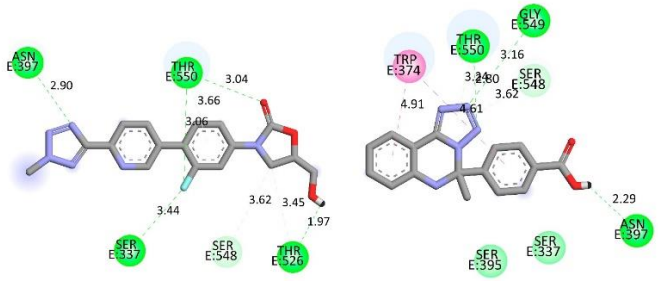

Fig. 2. Visual representation (2D) of the Tedizolid versus lead compound 12 showing bonds formation in the active site of penicillin binding protein 2X (PBD ID: 2ZC4). Pale green − van der Waals interaction or Carbon Hydrogen bond, green – conventional Hydrogen Bond, light green – π-Donor Hydrogen Bond, violet − hydrophobic π-π stacked and π-π T shaped bonds.

Hence, Tedizolid has formed 8 Hydrogen Bonds: 1 π-Donor (with THR550), 2 Carbon (THR526, and SER548), and 5 Conventional (THR526, THR550, ASN397, and SER337), along with two Halogen (Fluorine) ones. And substance 12 has formed 7 bonds, among which: 3 Conventional Hydrogen ones to GLY549, THR550, ASN397; 2 π-Donor Hydrogen Bonds also to THR550, and SER548; and two hydrophobic π-π stacked and π-π T shaped bonds to SER374.

Conclusions. Hence, results of molecular docking of 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]quinazolines towards 2QEX and 2ZC4 have shown, that substance 1, bearing 4-CH_3 group, 2 having 3-CF_3 on phenyl ring, 11, bearing 4-CN, and 12, having 4-COOH in phenyl ring along with 5-CH_3 for last two ones, have molecular structures to be promising antimicrobials. Summing up with ADME data, among the above listed substances, only 2 has violation of Lipinski rule for lipophilicity MLOGP>4.15 (4.21) and lead likeness violation of XLOGP3>3.5. All others are totally in agreement with Ghose, Veber, Egan, Muegge rules to be drug candidates.

Besides, up-to-date study of substance 12 in 50 μg/mL has showed its antifungal activity inhibiting growth of *C. albicans* at about 99% (Fig. 3), when only single smallest colonies were found by agar dilution method.

Fig. 3. Growth inhibition results (duplicate) of C. albicans by substance 12 in 50 μg/mL versus control.

And, promising *in vitro* antibacterial research is ongoing too.

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