Synthesis and Characterization of Novel *N*-(Phenyl, Benzyl, Hetaryl)-2-([1,2,4]Triazolo[1,5-*c*]Quinazolin-2-ylthio)Acetamides by Spectral Data, Antimicrobial Activity, Molecular Docking and QSAR Studies

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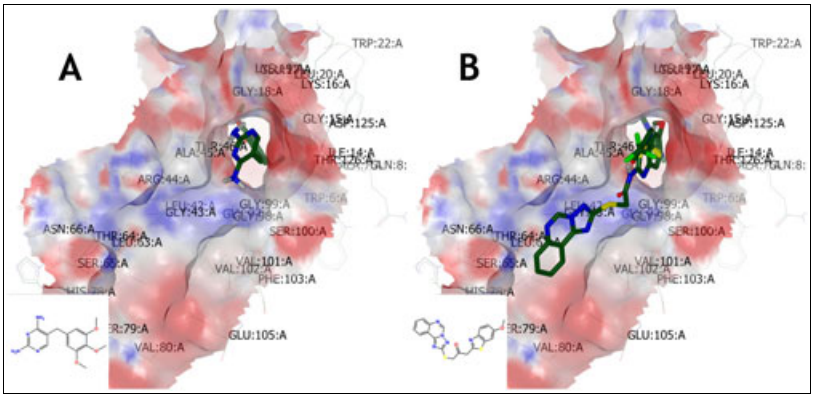

Enduring the novel effective antimicrobial agents search, *N*-(phenyl, benzyl, hetaryl)-2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetamides were synthesized, evaluated for structure (LC-MS, IR, ^{1}H-NMR spectra and elemental analysis), and investigated for antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Cronobacter sakazaki*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia*, and antifungal – against *Candida albicans*. *N*-(4-Fluorophenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetamide **3e** had the best minimum inhibition zones against *S. aureus* and *E. faecalis* and 3-{[([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetyl]amino}benzoic acid **3k** – against *E. coli*, still in lower concentration, than references. By the means of *in silico* molecular docking into the active sites of *E. faecalis* dihydrofolate reductase and *Enterobacter cloacae* MyrA, the possible activity mechanism was suggested. The quantitative structure–activity relationship model for antimicrobial activity prediction was calculated.

INTRODUCTION

According to the latest paper of Spellberg and Shlaes [1] about antibiotic resistance among the top areas of unmet medical treatment are extreme drug-resistant (XDR) and pan drug-resistant (PDR) Gram negative bacilli infection, common community-onset infections such as urinary tract infections and intra-abdominal infections caused by gram negative bacilli to all oral options, XDR/PDR tuberculosis, bloodstream, and bone infections caused by MRSA, and XDR/PDR gonorrhea. Thus, it is of undoubted interest, to synthesize and investigate for antimicrobial activity the novel antimicrobial agents as continuation of novel derivatives of [1,2,4]triazolo[1,5-*c*]quinazoline search, which were already proven to have high antifungal activity against *Candida ablicans*, namely ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetic and propionic acids, **A** [2], and antimicrobial activity against *Staphylococcus aureus* – potassium 2-heteroaryl-[1,2,4]triazolo[1,5-*c*]quinazoline-5-thiolates, **B** [3] (Fig. 1).

Considering the most active antimicrobial and antifungal compounds against *S. aureus*, *P. aeruginosa*, *K. pneumonia*, and *C. albicans*, unsubstituted, 2-methyl-, and 2-phenylquinazolin-4(3*H*)-thiones, **C** appeared to be the strongest (Fig. 1) [4], and 1-(2,5-dimethoxyphenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)ethanone was effective against *Escherichia coli*, *S. aureus*, *Cystopteris tenius* and *Aspergillus niger* [5]. The 2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)-*N*-butylpropanamide, *N*-(2-methoxyphenyl)-propanamide, and *N*,*N*-diethylacetamide **D** [6] also inhibited growth of *A niger*. Analyzing the literature data about other triazole and quinazoline substituted amides as antimicrobials, investigation of (*Z*)-*N*-(4-(3-(2,4-dichlorophenyl)-3-oxo-2-(1*H*-1,2,4-triazolo-1-yl) prop-1-enyl)phenyl)benzamides, **E** [7] showed that they inhibited the growth of fungi *Pellicularia sasakii*, *Gibberella zeae*, *Fusarium oxysporum*, *Cytospora mandshurica*, *Phytophthora infestans* and bacteria *Ralstonia solanacearum*. As well Metkonazol and Triadimefon [8] also had antifungal properties and exhibit

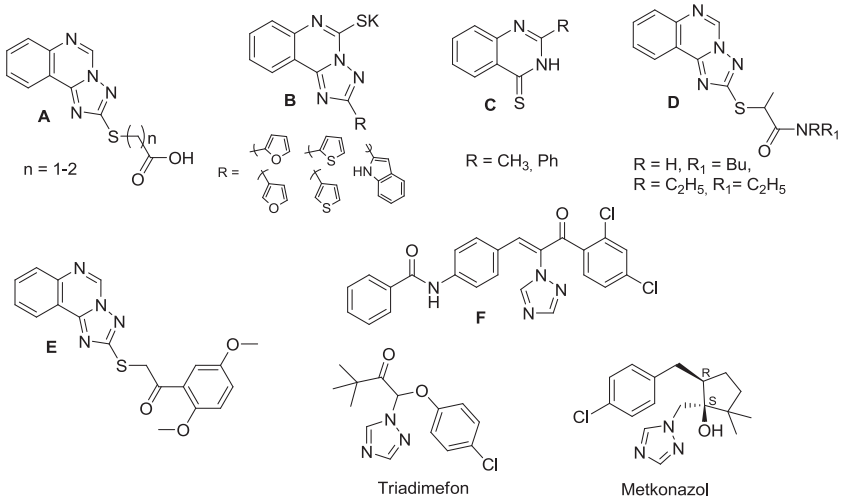

Figure 1. Structures of antimicrobial representatives of quinazolines, 1,2,4-triazoles and [1,2,4]triazolo[1,5-*c*]quinazolines.

antimicrobial activity against *Paralepetopsis sasakii*, *F. oxysporum*, *Corylus mandshurica*, and *Phytophthora infestans*.

Hence, it was decided to extend the series of amides among [1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio-derivatives for synthesis and screening for antimicrobial properties against the most common microbial pathogens by drug-like modeling with subsequent molecular docking studies and quantitative structure–activity relationship (QSAR) analysis.

EXPERIMENTAL

Chemistry. Melting points were determined in open capillary tubes in a Thiele's apparatus and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL Cube analyzer (Hanau, Germany). Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer (Ettlingen, Germany) using a module for measuring attenuated total reflection. ^{1}H NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 spectrometer (Palo Alto, CA) with $SiMe_4$ as internal standard in DMSO-d_6 + CCl_4 solution. LC-MS were recorded using chromatography/mass spectrometric system which consisted of high-performed liquid chromatograph "Agi1ent 1100 Series" equipped with diode-matrix and mass-selective detector "Agi1ent LC/MSD SL" (atmospheric pressure chemical ionization – APCI (Palo Alto, CA)). The purity of all obtained compounds was checked by ^{1}H NMR and LC–MS.

Potassium [1,2,4]triazolo[1,5-*c*]quinazolin-2-thiole **1** was synthesized according to the reported procedure [9]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

Examples of **1d**, **2c**, **3b** spectra are given in the Appendix S1.

General procedure for the synthesis of 2-([1,2,4]triazolo [1,5-*c*]quinazolin-2-ylsulfanyl)acetamides. To solution of corresponding halogenoacetamide (0.005 mol) in 10 mL of propan-2-ol the solution of potassium [1,2,4]triazolo [1,5-*c*]quinazolin-5-thiole (0.005 mol) in 10 mL of water was added. The mixture was refluxed for 2 h and then cooled down. The precipitate was filtered, washed with water, dried and recrystallized from propan-2-ol.

N-(2-chlorobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio) acetamide (1a). Yellow solid. Yield: 31.0%, mp. 150–152°C. IR (cm^{-1}) 3391, 3261, 3202, 3056, 2949, 2918, 2867, 1693, 1622, 1605, 1553, 1518, 1481, 1455, 1400, 1385, 1375, 1359, 1308, 1258, 1212, 1161, 1141, 1107, 1072, 1054, 967, 909, 834, 785, 768, 741, 717, 694, 675, 621. ^{1}H NMR δ (ppm) 9.36 (s, 1H, H-5), 8.65 (br. s, 1H, NH), 8.40 (d, J = 7.8 Hz, 1H, H-10), 8.02 (d, J = 8.0 Hz, 1H, H-7), 7.90 (t, J = 7.7 Hz, 1H, H-8), 7.77 (t, J = 7.5 Hz, 1H, H-9), 7.35 (d, J = 7.6 Hz, 1H, Ph-3), 7.31 (d, J = 7.6 Hz, 1H, Ph-6), 7.17–7.12 (m, J = 7.6 Hz, 2H, Ph-4,5), 4.39 (d, J = 5.6 Hz, 2H, $HNCH_2$), 4.08 (s, 2H, SCH_2). LC-MS m/z = 384 $[M+H]^+$. *Anal.* Calcd for $C_{18}H_{14}ClN_5OS$: S, 56.32; H,3.68; N, 18.24; S, 8.35. Found: S, 56.33; H, 3.70; N, 18.26; S, 8.36.

N-(3-chlorobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio) acetamide (1b). Light brown solid. Yield: 48.5%, mp. 265–267°C. IR (cm^{-1}) 3286, 3053, 2923, 2852, 1698, 1670, 1620, 1599, 1575, 1539, 1517, 1494, 1477, 1454, 1428, 1397, 1385, 1357, 1339, 1313, 1285, 1249, 1203, 1156, 1127, 1105, 1078, 1039, 1017, 977, 939, 904, 863, 848, 774, 743, 717, 700, 670. ^{1}H NMR δ (ppm) 9.34 (s, 1H, H-5), 8.67 (br. s, 1H, NH), 8.58 (d, J = 7.2 Hz, 1H,

Ph-6), 8.39 (d, J = 7.7 Hz, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.88 (t, J = 7.4 Hz, 1H, H-8), 7.78 (t, J = 7.5 Hz, 1H, H-9), 7.27–7.10 (m, 4H, Ph-2,4), 4.36–4.28 (m, 2H, $NHCH_2$), 4.05 (s, 2H, SCH_2). LC-MS m/z = 384 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{14}ClN_5OS$: S, 56.32; H, 3.68; N, 18.24; S, 8.35. Found: S, 56.34; H, 3.67; N. 18.25; S, 8.33.

N-(4-chlorobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio) acetamide (1c). Light brown solid. Yield: 35.0%, mp. 189–190°C. IR (cm^{-1}) 3315, 3055, 2918, 2850, 1669, 1621, 1603, 1553, 1517, 1491, 1476, 1454, 1397, 1383, 1374, 1358, 1310, 1297, 1257, 1210, 1191, 1177, 1104, 1088, 1050, 1014, 962, 938, 904, 879, 833, 814, 800, 783, 717, 668, 645, 622. ^{1}H NMR δ (ppm) 9.36 (s, 1H, H-5), 8.64 (br. s, 1H, NH), 8.37 (d, J = 7.8 Hz, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.89 (t, J = 7.5 Hz, 1H, H-8), 7.77 (t, J = 7.4 Hz, 1H, H-9), 7.25 (d, J = 8.1 Hz, 2H, Ph-3,5), 7.20 (d, J = 8.1 Hz, 2H, Ph-2,6), 4.31 (d, J = 5.7 Hz, 2H, $HNCH_2$), 4.04 (s, 2H, SCH_2). LC-MS m/z = 384 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{14}ClN_5OS$: S, 56.32; H, 3.68; N, 18.24; S, 8.35. Found: S, 56.33; H, 3.71; N, 18.25; S, 8.37.

N-(2-bromobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (1d). White solid. Yield: 45.5%, mp. 147–145°C. IR (cm^{-1}) 3299, 3056, 2918, 1681, 1666, 1623, 1607, 1564, 1555, 1537, 1518, 1478, 1455, 1440, 1422, 1400, 1388, 1359, 1326, 1313, 1269, 1213, 1195, 1130, 1106, 1069, 1046, 1028, 948, 931, 907, 782, 768, 753, 716, 696, 673, 660, 647, 614. ^{1}H NMR δ (ppm) 9.37 (s, 1H, H-5), 8.62 (br. s, 1H, NH), 8.41 (d, J = 7.9 Hz, 1H, H-10), 8.03 (d, J = 8.1 Hz, 1H, H-7), 7.90 (t, J = 7.7 Hz, 1H, H-8), 7.78 (t, J = 7.5 Hz, 1H, H-9), 7.48 (d, J = 7.7 Hz, 1H, Ph-3), 7.34 (d, J = 7.3 Hz, 1H, Ph-6), 7.19 (t, J = 7.1 Hz, 1H, Ph-4), 7.10 (t, J = 7.5 Hz, 1H, Ph-5), 4.37 (d, J = 5.7 Hz, 2H, $HNCH_2$), 4.09 (s, 2H, SCH_2). LC-MS m/z = 429 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{14}BrN_5OS$: S, 50.48; H, 3.29; N, 16.35; S, 7.49. Found: S, 50.50; H, 3.28; N, 16.36; S, 7.55.

N-(4-bromobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio) acetamide (1e). Light brown solid. Yield: 75.0%, mp. 141–143°C. IR (cm^{-1}) 3748, 3321, 2924, 2851, 1672, 1619, 1603, 1531, 1517, 1487, 1475, 1454, 1395, 1382, 1373, 1310, 1296, 1258, 1192, 1176, 1103, 1069, 1051, 1011, 904, 798, 783, 718, 627. ^{1}H NMR δ (ppm) 9.36 (s, 1H, H-5), 8.64 (br. s, 1H, NH), 8.37 (d, J = 7.8 *Hz*, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.89 (t, J = 7.5 Hz, 1H, H-8), 7.77 (t, J = 7.4 Hz, 1H, H-9), 7.25 (d, J = 8.1 Hz, 2H, Ph-3,5), 7.20 (d, J = 8.1 Hz, 2H, Ph-2,6), 4.31 (d, J = 5.7 Hz, 2H, $HNCH_2$), 4.04 (s, 2H, SCH_2). LC-MS m/z = 429 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{14}BrN_5OS$: S, 50.48; H, 3.29; N, 16.35; S, 7.49. Found: S, 50.50; H, 3.30; N. 16.37; S, 7.50.

N-(2-fluorobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio) acetamide (1f). Orange solid. Yield: 45.5%, mp. 157–159°C. IR (cm^{-1}) 3834, 3745, 3617, 3315, 2917, 1756, 1670, 1616, 1551, 1522, 1477, 1454, 1388, 1311, 1258, 1203, 1100, 1076, 1017, 979, 938, 905, 846, 774, 752, 714, 638. ^{1}H NMR δ (ppm) 9.35 (s, 1H, H-5), 8.61 (br. s, 1H, NH), 8.47 (s, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.90 (t, J = 7.0 Hz, 1H, H-8), 7.77 (t, J = 7.6 Hz, 1H, H-9), 7.19 (d, J = 5.9 Hz, 2H, Ph-3,6), 7.01 (t, J = 7.7 Hz, 2H, Ph-4,5), 4.36 (d, J = 5.7 Hz, 2H, $HNCH_2$), 4.06 (s, 2H, SCH_2). LC-MS m/z = 368 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{14}FN_5OS$: S, 58.84; H, 3.84; N, 19.06; S, 8.73. Found: S, 58.84; H, 3.86; N, 19.08; S, 8.73.

N-(3-fluorobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio) acetamide (1g). Light brown solid. Yield: 40.3%, mp. 171–173°C. IR (cm^{-1}) 3327, 3290, 3064, 2909, 1668, 1620, 1604, 1591, 1551, 1516, 1479, 1453, 1397, 1385, 1360, 1317, 1265, 1252, 1204, 1137, 1105, 1075, 1024, 1005, 981, 950, 929, 904, 859, 778, 743, 718, 683, 669, 644, 617. ^{1}H NMR δ (ppm) 9.34 (s, 1H, H-5), 8.66 (br. s, 1H, NH), 8.40 (d, J = 8.2 Hz, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.89 (t, J = 7.3 Hz, 1H, H-8), 7.77 (t, J = 7.4 Hz, 1H, H-9), 7.25–7.20 (m, 1H, Ph-5), 7.06 (d, J = 7.6 Hz, 1H, Ph-4), 7.01 (d, J = 9.3 *Hz*, 1H, Ph-5), 6.88 (s, 1H, Ph-2), 4.34 (d, J = 5.8 Hz, 2H, $NHCH_2$), 4.05 (s, 2H, SCH_2). LC-MS m/z = 368 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{14}FN_5OS$: S, 58.84; H, 3.84; N, 19.06; S, 8.73. Found: S, 58.86; H, 3.85; N, 19.08; S, 8.74.

N-(4-fluorobenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio) acetamide (1h). Light brown solid. Yield: 49.5%, mp. 186–188°C. IR (cm^{-1}) 3321, 3048, 2920, 2850, 1667, 1620, 1603, 1547, 1506, 1476, 1453, 1445, 1413, 1395, 1384, 1357, 1315, 1263, 1254, 1226, 1195, 1152, 1104, 1092, 1016, 982, 963, 937, 903, 878, 849, 818, 805, 781, 761, 718, 643. ^{1}H NMR δ (ppm) 9.36 (s, 1H, H-5), 8.64 (br. s, 1H, NH), 8.37 (d, J = 7.8 Hz, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.89 (t, J = 7.5 Hz, 1H, H-8), 7.77 (t, J = 7.4 Hz, 1H, H-9), 7.25 (d, J = 8.1 Hz, 2H, Ph-3,5), 6.35 (d, J = 8.1 Hz, 2H, Ph-2,6), 4.31 (d, J = 5.7 Hz, 2H, $HNCH_2$), 4.04 (s, 2H, SCH_2). LC-MS m/z = 368 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{14}FN_5OS$: S, 58.84; H, 3.84; N, 19.06; S, 8.73. Found: S, 56.83; H, 3.85; N, 19.08; S, 8.71.

N-(2-(fluoromethyl)benzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (1i). Yellow solid. Yield: 43.5%, mp. 143–145°C. IR (cm^{-1}) 3276, 3066, 2931, 2856, 1734, 1717, 1683, 1653, 1635, 1624, 1606, 1576, 1558, 1550, 1533, 1520, 1508, 1498, 1478, 1456, 1436, 1398, 1372, 1357, 1310, 1265, 1246, 1220, 1192, 1162, 1116, 1071, 1058, 1033, 981, 960, 934, 900, 886, 868, 804, 767, 717, 673, 644, 626. ^{1}H NMR δ (ppm) 9.37 (s, 1H, H-5), 8.62 (br. s, 1H, NH), 8.41 (d, J = 7.9 Hz, 1H, H-10), 8.03 (d, J = 8.1 Hz, 1H, H-7), 7.90 (t, J = 7.7 Hz, 1H, H-8), 7.78 (t, J = 7.5 Hz, 1H, H-9), 7.48 (d, J = 7.7 Hz, 1H, Ph-3), 7.34 (d, J = 7.3 Hz, 1H, Ph-6), 7.19 (t, J = 7.1 Hz, 1H, Ph-4), 7.10 (t, J = 7.5 Hz, 1H, Ph-5), 4.37 (d, J = 5.7 Hz, 2H,

$HNCH_2$), 4.09 (s, 2H, SCH_2). LC-MS $m/z = 418$ $[M+H]^+$. *Anal*. Calcd for $C_{19}H_{14}F_3N_5OS$: S, 54.67; H, 3.38; N, 16.78; S, 7.68. Found: S, 54.65; H, 3.40; N, 16.80; S, 7.69.

N-(4-(fluoromethyl)benzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (1j). Yellow solid. Yield: 50.3%, mp. 178–180°C. IR (cm^{-1}) 3868, 3856, 3746, 3736, 3724, 3610, 3336, 3310, 3064, 2924, 2854, 1711, 1690, 1673, 1650, 1621, 1564, 1551, 1535, 1515, 1502, 1479, 1453, 1418, 1397, 1386, 1323, 1266, 1254, 1212, 1193, 1158, 1107, 1066, 1018, 982, 938, 905, 817, 783, 776, 753, 717, 689, 668, 636. ^{1}H NMR δ (ppm) 9.37 (s, 1H, H-5), 8.73 (br. s, 1H, NH), 8.40 (d, $J = 7.8$ Hz, 1H, H-10), 8.03 (d, $J = 8.1$ Hz, 1H, H-7), 7.90 (d, $J = 6.8$ Hz, 1H, H-8), 7.78 (d, $J = 7.4$ Hz, 1H, H-9), 7.55–7.35 (m, 4H, Ph-2,3,5,6), 4.41 (d, $J = 5.5$ Hz, 1H, $HNCH_2$), 4.07 (s, 2H, SCH_2). LC-MS $m/z = 418$ $[M+H]^+$. *Anal*. Calcd for $C_{19}H_{14}F_3N_5OS$: S, 54.67; H, 3.38; N, 16.78; S, 7.68. Found: S, 54.65; H, 3.40; N, 16.81; S, 7.80.

N-(3-methoxybenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (1k). Light green solid. Yield: 51.5%, mp. 154–156°C. IR (cm^{-1}) 3275, 3062, 2955, 2921, 2851, 1646, 1624, 1602, 1540, 1522, 1493, 1473, 1453, 1429, 1397, 1372, 1353, 1305, 1265, 1214, 1182, 1167, 1103, 1084, 1070, 1040, 1003, 963, 926, 915, 899, 888, 841, 795, 776, 747, 718, 692, 641, 623. ^{1}H NMR δ (ppm) 9.34 (s, 1H, H-5), 8.56 (br. s, 1H, NH), 8.39 (d, $J = 7.9$ Hz, 1H, H-10), 8.02 (d, $J = 7.7$ Hz, 1H, H-7), 7.89 (t, $J = 7.6$ Hz, 1H, H-8), 7.77 (t, $J = 7.3$ Hz, 1H, H-9), 7.10 (t, $J = 7.8$ Hz, 1H, Ph-5), 6.79 (d, $J = 7.6$ Hz, 1H, Ph-4), 6.76 (s, 1H, Ph-2), 6.67 (d, $J = 8.3$ Hz, 1H, Ph-6), 4.30 (d, $J = 5.3$ Hz, 2H, $NHCH_2$), 4.04 (s, 2H, SCH_2), 3.68 (s, 3H, OCH_3). LC-MS $m/z = 380$ $[M+H]^+$. *Anal*. Calcd for $C_{19}H_{17}N_5O_2S$: S, 60.14; H, 4.52; N, 18.46; S, 8.45. Found: S, 60.12; H, 4.51; N, 18.45; S, 8.47.

N-(4-methoxybenzyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (1l). Orange solid. Yield: 55.0%, mp. 170–172°C. IR (cm^{-1}) 3309, 2950, 2917, 2849, 1666, 1621, 1605, 1553, 1511, 1477, 1454, 1396, 1385, 1318, 1302, 1241, 1211, 1197, 1171, 1103, 1029, 962, 903, 804, 774, 717, 682, 667, 642, 625. ^{1}H NMR δ (ppm) 9.34 (s, 1H, H-5), 8.57 (br. s, 1H, NH), 8.39 (d, $J = 7.9$ Hz, 1H, H-10), 8.03 (d, $J = 7.8$ Hz, 1H, H-7), 7.89 (t, $J = 7.6$ Hz, 1H, H-8), 7.78 (t, $J = 7.3$ Hz, 1H, H-9), 7.13 (d, $J = 7.6$ Hz, 1H, Ph-3,5), 6.72 (d, $J = 7.6$ Hz, 1H, H-7, Ph-2,6), 4.25 (d, $J = 5.3$ Hz, 2H, $NHCH_2$), 4.02 (s, 2H, SCH_2), 3.67 (s, 3H, OCH_3). LC-MS $m/z = 380$ $[M+H]^+$. *Anal*. Calcd for $C_{19}H_{17}N_5O_2S$: S, 60.14; H, 4.52; N, 18.46; S, 8.45. Found: S, 60.12; H, 4.51; N, 18.45; S, 8.47.

N-(5-butyl-1,3,4-thiadiazol-2-yl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide solid. (2a). Brown solid. Yield: 90.4%, mp. 176–178°C. IR (cm^{-1}) 3391, 3261, 3202, 3056, 2949, 2918, 1624, 1600, 1553, 1520, 1481, 1445, 1400, 1384, 1375, 1357, 1332, 1309, 1258, 1229, 1162, 1153, 1107, 1096, 1052, 993, 966, 934, 907, 871, 818, 761, 752, 737, 718, 658, 618. ^{1}H NMR δ (ppm) 12.76 (br. s, 1H, NH), 9.36 (s, 1H, H-5), 8.39 (d, $J = 7.9$ Hz, 1H, H-10), 8.01 (d, $J = 8.1$ Hz, 1H, H-7), 7.88 (t, $J = 7.6$ Hz, 1H, H-8), 7.76 (t, $J = 7.4$ Hz, 1H, H-9), 4.34 (s, 2H, SCH_2), 2.83 (t, $J = 6.7$ Hz, 2H, $CH_2C_3H_7$), 1.23 (s, 3H, CH_3), 1.01–0.96 (m, 4H, $(CH_2)_2CH_3$). LC-MS $m/z = 400$ $[M+H]^+$. *Anal*. Calcd for $C_{19}H_{23}N_7OS_2$:S, 57.11; H, 4.29; N, 24.54; S, 16.05. Found: S, 57.12; H, 4.30; N, 24.56; S, 16.07.

N-(6-methoxy-1,3-benzothiazol-2-yl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (2b). Brown solid. Yield: 86.8%, mp. 200–202°C. IR (cm^{-1}) 3215, 3102, 3060, 3019, 2963, 2923, 1703, 1622, 1606, 1574, 1561, 1516, 1484, 1471, 1455, 1431, 1402, 1375, 1362, 1310, 1255, 1227, 1187, 1155, 1113, 1059, 1031, 993, 984, 907, 836, 811, 776, 753, 717, 662, 648, 615. ^{1}H NMR δ (ppm) 12.51 (br. s, 1H, NH), 9.38 (s, 1H, H-5), 8.42 (d, $J = 7.9$ Hz, 1H, H-10), 8.01 (d, $J = 6.6$ Hz, 1H, H-7), 7.87 (t, $J = 7.5$ Hz, 1H, H-8), 7.77 (t, $J = 7.6$ Hz, 1H, H-9), 7.59 (d, $J = 8.5$ Hz, 1H, Ph-8), 7.38 (s, 1H, Ph-5), 6.95 (d, $J = 7.3$ Hz, 1H, Ph-7), 4.37 (s, 2H, SCH_2), 1.24 (s, 3H, OCH_3). LC-MS $m/z = 437$ $[M+H]^+$. *Anal*. Calcd for $C_{20}H_{16}N_6O_2S_2$: S, 54.02; H, 3.34; N, 19.89; S, 15.18. Found: S, 59.84; H, 3.41; N, 21.77; S, 15.17.

N-(6-chloro-1,3-benzothiazol-2-yl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (2c). White solid. Yield: 60.0%, mp. 271–272°C. IR (cm^{-1}) 3201, 3057, 3037, 3004, 2959, 2917, 2854, 1697, 1624, 1600, 1553, 1520, 1481, 1445, 1400, 1385, 1375, 1357, 1322, 1309, 1258, 1229, 1162, 1152, 1107, 1096, 1052, 993, 966, 934, 907, 871, 818, 770, 761, 751, 737, 718, 687, 658, 619. ^{1}H NMR δ (ppm) 12.81 (br. s, 1H, NH), 9.37 (s, 1H, H-5), 8.39 (d, $J = 7.8$ Hz, 1H, H-10), 8.02 (d, $J = 8.1$ Hz, 1H, H-7), 7.88 (t, $J = 7.6$ Hz, 1H, H-8), 7.76 (t, $J = 7.4$ Hz, 1H, H-9), 7.35–7.21 (m, 3H, Ph-5,7,8), 4.36 (s, 2H, SCH_2). LC-MS $m/z = 427$ $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{11}ClN_6OS_2$: S, 50.64; H, 2.60; N, 19.69; S, 15.02. Found: S, 50.65; H, 2.61; N, 19.70; S, 15.03.

N-(4-Chlorophenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3a). Brown solid. Yield: 59.0%, mp. 172–174°C. IR (cm^{-1}) 3309, 2952, 2921, 2851, 1682, 1622, 1593, 1531, 1516, 1473, 1465, 1454, 1436, 1420, 1396, 1368, 1350, 1314, 1301, 1277, 1262, 1248, 1235, 1189, 1171, 1125, 1102, 1050, 1036, 957, 895, 881, 854, 777, 763, 717, 673, 641. ^{1}H NMR δ (ppm) 9.64 (br. s, 1H, NH), 9.40 (s, 1H, H-5), 8.44 (d, $J = 7.8$ Hz, 1H, H-10), 8.05–8.02 (m, 2H, Ph-5, H-7), 7.90 (t, $J = 7.3$ Hz, 1H, H-8), 7.78 (t, $J = 7.3$ Hz, 1H, H-9), 7.36 (d, $J = 7.6$ Hz, 1H, Ph-3), 7.26 (t, $J = 7.6$ Hz, 1H, Ph-4), 7.09 (d, $J = 7.5$ Hz, 1H, Ph-6), 4.28 (s, 2H, SCH_2). LC-MS $m/z = 370$ $[M+H]^+$. *Anal*. Calcd for $C_{17}H_{12}ClN_5O$: S, 55.21; H, 3.27; N, 18.94; S, 8.67. Found: S, 55.20; H, 3.25; N, 18.95; S, 8.65.

N-(2-bromophenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3b). Brown solid. Yield: 62.0%, mp. 186–188°C. IR (cm^{-1}) 3307, 2983, 2919, 2850, 1674, 1621, 1604, 1588, 1576, 1516, 1475, 1453, 1433, 1392, 1374, 1352, 1318, 1300, 1273, 1261, 1240, 1207, 1169, 1154, 1106, 1045, 1023, 956, 896, 878, 851, 778, 763, 717, 689, 653. ^{1}H NMR δ (ppm) 9.53 (br. s, 1H, NH), 9.42 (s, 1H, H-5), 8.45 (d, J = 7.8 Hz, 1H, H-10), 8.03 (d, J = 8.3 Hz, 1H, H-7), 7.95 (d, J = 8.4 Hz, 1H, Ph-3), 7.90 (t, J = 7.7 Hz, 1H, H-8), 7.78 (t, J = 7.3 Hz, 1H, H-9), 7.53 (d, J = 7.9 Hz, 1H, Ph-6), 7.31 (d, J = 7.6 Hz, 1H, Ph-4), 7.03 (t, J = 7.5 Hz, 1H, Ph-5), 4.26 (s, 2H, SCH_2). LC-MS m/z = 415 $[M+H]^+$. *Anal*. Calcd for $C_{17}H_{12}BrN_5OS$: S, 49.29; H, 2.92; N, 16.90; S, 7.74. Found: S, 49.28; H, 2.94; N, 16.92; S, 7.71.

N-(3-bromophenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3c). Light green solid. Yield: 60.0%, mp. 125–127°C. IR (cm^{-1}) 3255, 3181, 3122, 3064, 2993, 2918, 2850, 1682, 1668, 1621, 1587, 1535, 1516, 1478, 1454, 1401, 1385, 1333, 1307, 1283, 1253, 1187, 1163, 1146, 1131, 1092, 1071, 1049, 995, 981, 900, 869, 779, 765, 747, 715, 681, 658, 623. ^{1}H NMR δ (ppm) 10.40 (br. s, 1H, NH), 9.39 (s, 1H, H-5), 8.42 (d, J = 7.9 Hz, 1H, H-10), 8.02 (d, J = 8.2 Hz, 1H, H-7), 7.89 (dd, J = 7.1 Hz, 2H, H-8, Ph-2), 7.77 (t, J = 7.4 Hz, 1H, H-9), 7.21–7.16 (m, 3H, Ph-4-6), 4.24 (s, 2H, SCH_2). LC-MS m/z = 415 $[M+H]^+$. *Anal*. Calcd for $C_{17}H_{12}BrN_5OS$: S, 49.29; H, 2.92; N, 16.90; S, 7.74. Found: S, 49.31; H, 2.93; N, 16.88; S, 7.75.

N-(2-fluorophenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3d). White solid. Yield: 52.7%, mp. 114–116° C. IR (cm^{-1}) 3270, 3217, 3135, 3048, 2919, 2850, 1684, 1621, 1603, 1545, 1515, 1488, 1476, 1454, 1395, 1372, 1355, 1330, 1301, 1266, 1251, 1233, 1190, 1132, 1101, 1034, 962, 899, 841, 807, 768, 742, 717, 697, 684, 643. ^{1}H NMR δ (ppm) 10.03 (br. s, 1H, NH), 9.39 (s, 1H, H-5), 8.44 (d, J = 7.7 Hz, 1H, H-10), 8.02 (t, J = 7.0 Hz, 1H, H-7), 7.90 (t, J = 7.8 Hz, 1H, H-8), 7.79 (t, J = 7.7 Hz, 1H, H-9), 7.13–7.08 (m, 4H, Ph-3-6), 4.28 (s, 2H, SCH_2). LC-MS m/z = 354 $[M+H]^+$. *Anal*. Calcd for $C_{17}H_{12}FN_5OS$: S, 57.78; H, 3.42; N, 19.82; S, 9.07. Found: S, 57.79; H, 3.45; N, 19.80; S, 9.09.

N-(4-fluorophenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3e). Light brown solid. Yield: 56.8%, mp. 237–239°C. IR (cm^{-1}) 3130, 3084, 2919, 2851, 2772, 2652, 2577, 1658, 1650, 1632, 1620, 1563, 1556, 1537, 1530, 1504, 1461, 1411, 1384, 1329, 1289, 1266, 1225, 1210, 1156, 1137, 1124, 1090, 1061, 1024, 978, 958, 902, 868, 856, 829, 803, 771, 737, 704, 675, 641. ^{1}H NMR δ (ppm) 10.52 (br. s, 1H, NH), 9.37 (s, 1H, H-5), 8.43 (d, J = 7.8 Hz, 1H, H-10), 8.03 (d, J = 8.1 Hz, 1H, H-7), 7.89 (t, J = 7.5 Hz, 1H, H-8), 7.77 (t, J = 7.5 Hz, 1H, H-9), 7.21 (d, J = 8.0 Hz, 2H, Ph-3,5), 7.20 (d, J = 8.1 Hz, 2H, Ph-2,6), 4.15 (s, 2H, SCH_2). LC-MS m/z = 354 $[M+H]^+$. *Anal*. Calcd for $C_{17}H_{12}FN_5OS$: S, 57.78; H, 3.42; N, 19.82; S, 9.07. Found: S, 57.75; H, 3.46; N, 19.82; S, 9.08.

N-(2-(trifluoromethyl)phenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3f). Light brown solid. Yield: 45.0%, mp. 169–171°C. IR (cm^{-1}) 3200, 3138, 3065, 3021, 2965, 2940, 2909, 2882, 2830, 1671, 1656, 1624, 1601, 1547, 1537, 1521, 1491, 1480, 1468, 1451, 1426, 1395, 1374, 1353, 1320, 1308, 1284, 1261, 1247, 1220, 1178, 1160, 1153, 1081, 1039, 996, 965, 899, 891, 850, 790, 768, 734, 718, 687, 642. ^{1}H NMR δ (ppm) 10.20 (br. s, 1H, NH), 9.40 (s, 1H, H-5), 8.43 (d, J = 8.0 Hz, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.89 (t, J = 7.7 Hz, 1H, H-8), 7.78 (t, J = 7.5 *Hz*, 1H, H-9), 7.34–7.30 (m, 1H, Ph-6), 7.13 (dd, J = 15.8, 8.0 Hz, 2H, Ph-4,5), 6.55 (d, J = 7.3 Hz, 1H, Ph-3), 4.24 (s, 2H, SCH_2). LC-MS m/z = 404 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{12}F_3N_5OS$: S, 53.60; H, 3.00; N, 17.36; S, 7.95. Found: S, 53.62; H, 3.01; N, 17.34; S, 7.96.

N-(4-(trifluoromethyl)phenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3g). Yellow solid. Yield: 64.0%, mp. 220–222°C. IR (cm^{-1}) 3324, 3063, 1675, 1620, 1602, 1526, 1513, 1483, 1406, 1396, 1377, 1360, 1335, 1302, 1266, 1248, 1185, 1160, 1113, 1068, 1019, 971, 904, 866, 835, 783, 771, 715, 656, 612. ^{1}H NMR δ (ppm) 10.58 (br. s, 1H, NH), 9.40 (s, 1H, H-5), 8.43 (d, J = 7.6 Hz, 1H, H-10), 8.02 (d, J = 8.1 Hz, 1H, H-7), 7.89 (t, J = 7.8 Hz, 1H, H-8), 7.79 (dd, J = 15.8, 8.0 Hz, 3H, H-9, Ph-3,5), 7.55 (d, J = 8.3 Hz, 2H, Ph-2,4), 4.28 (s, 2H, SCH_2). LC-MS m/z = 404 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{12}F_3N_5OS$: S, 54.67; H, 3.38; N, 16.78; S, 7.68. Found: S, 54.65; H, 3.40; N, 16.81; S, 7.80.

N-(4-methylphenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3h). Yellow solid. Yield: 61.0%, mp. 192–194°C. IR (cm^{-1}) 3290, 3056, 2918, 2852, 1662, 1621, 1601, 1539, 1515, 1495, 1475, 1452, 1393, 1374, 1356, 1322, 1303, 1263, 1247, 1216, 1182, 1167, 1103, 1075, 1040, 1018, 1003, 985, 964, 937, 902, 877, 839, 814, 792, 772, 747, 717, 691, 641. ^{1}H NMR δ (ppm) 10.11 (br. s, 1H, NH), 9.39 (s, 1H, H-5), 8.42 (d, J = 7.8 Hz, 1H, H-10), 8.02 (d, J = 8.0 Hz, 1H, H-7), 7.89 (t, J = 6.9 Hz, 1H, H-8), 7.78 (t, J = 7.3 Hz, 1H, H-9), 7.46 (d, J = 7.7 Hz, 2H, Ph-2,6), 7.04 (d, J = 8.0 Hz, 1H, Ph-3,5), 4.23 (s, 2H, SCH_2), 2.29 (s, 3H, CH_3). LC-MS m/z = 350 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{15}N_5OS$: S, 61.87; H, 4.33; N, 4.58; S, 9.18. Found: S, 61.88; H, 4.35; N. 4.60; S, 9.20.

N-(4-methoxyphenyl)-2-([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetamide (3i). Light green solid. Yield: 68.1%, mp. 214–216°C. IR (cm^{-1}) 3298, 3045, 2977, 2927, 2840, 1660, 1616, 1601, 1541, 1510, 1475, 1451, 1412, 1389, 1373, 1356, 1330, 1301, 1247, 1235, 1179, 1166, 1104, 1033, 983, 960, 935, 902, 878, 831, 795, 769, 717, 694, 654, 641. ^{1}H NMR δ (ppm) 10.06 (br. s, 1H, NH), 9.39

(s, 1H, H-5), 8.42 (d, J = 7.7 Hz, 1H, H-10), 8.01 (d, J = 7.9 *Hz*, 1H, H-7), 7.88 (t, J = 7.4 Hz, 1H, H-8), 7.77 (t, J = 7.4 Hz, 1H, H-9), 7.50 (d, J = 8.5 Hz, 2H, Ph-2,6), 6.79 (d, J = 8.4 Hz, 2H, Ph-3,5), 4.21 (s, 2H, SCH_2), 3.74 (s, 3H, OCH_3). LC-MS m/z = 366 $[M+H]^+$. *Anal*. Calcd for $C_{17}H_{12}N_5O_2S$: S, 58.28; H, 3.45; N, 19.99; S, 9.15. Found: S, 58.30; H, 3.47; N. 20.01; S, 9.16.

2-{[([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetyl]amino} benzoic acid (3j). White solid. Yield: 64.5%, mp. 234–236°C. IR (cm^{-1}) 2917, 2851, 1842, 1695, 1668, 1589, 1520, 1474, 1450, 1409, 1385, 1353, 1308, 1265, 1248, 1217, 1163, 1107, 1093, 1050, 959, 930, 910, 803, 762, 737, 715, 696, 661, 651. ^{1}H NMR δ (ppm) 13.32 (br. s, 1H, OH), 11.95 (br. s, 1H, NH), 9.39 (s, 1H, H-5), 8.43 (d, J = 7.8 Hz, 1H, H-10), 8.18 (d, J = 6.1 Hz, 1H, Ph-3), 8.01 (d, J = 8.0 Hz, 1H, H-7), 7.87 (d, J = 6.1 Hz, 2H, H-8), 7.77 (t, J = 7.5 Hz, 1H, H-9), 7.63 (t, J = 7.3 Hz, 1H, Ph-4), 7.34 (t, J = 7.8 Hz, 1H, Ph-5), 7.35 (d, J = 7.8 Hz, 1H, Ph-6), 4.21 (s, 2H, SCH_2). LC-MS m/z = 380 $[M+H]^+$. *Anal*. Calcd for $C_{19}H_{15}N_5O_2S$: S, 56.98; H, 3.45; N,18.46; S, 8.45. Found: S, 56.99; H, 3.45; N. 18.41; S, 8.45.

3-{[([1,2,4]triazolo[1,5-c]quinazolin-2-ylthio)acetyl]amino} benzoic acid (3k). White solid. Yield: 69.0%, mp. 242–244°C. IR (cm^{-1}) 3011, 2898, 1705, 1680, 1646, 1613, 1567, 1556, 1512, 1488, 1477, 1454, 1437, 1393, 1373, 1334, 1304, 1254, 1240, 1225, 1146, 1104, 1082, 987, 956, 917, 900, 892, 867, 807, 785, 766, 749, 712, 676, 653. ^{1}H NMR δ (ppm) 12.61 (br. s, 1H, OH), 10.42 (br. s, 1H, NH), 9.38 (s, 1H, H-5), 8.42 (d, J = 7.7 Hz, 1H, H-10), 8.18 (s, 1H, Ph-2), 8.01 (d, J = 8.0 Hz, 1H, H-7), 7.87 (d, J = 6.2 Hz, 2H, H-8, Ph-6), 7.77 (t, J = 7.5 Hz, 1H, H-9), 7.63 (d, J = 7.3 Hz, 1H, Ph-4), 7.35 (t, J = 7.7 Hz, 1H, Ph-5), 4.25 (s, 2H, SCH_2). LC-MS m/z = 380 $[M+H]^+$. *Anal*. Calcd for $C_{18}H_{13}N_5O_3S$: S, 56.98; H, 3.45; N,18.46; S, 8.45. Found: S, 57.01; H, 3.46; N. 18.48; S, 8.47.

Quantitative structure–activity relationship-modeling. Firstly, all structures were built by MarvinSketch 6.3.0 [10]. Then, they were preliminary optimized by program HyperChem 8.0.8 using molecular mechanical MM+ algorithm combined with semi-empirical PM3 molecular modeling method with a maximum number of cycles and Polak-Ribiere (Conjugate Gradient) algorithm. Molecular mechanics have been used to produce more realistic geometry values for the majority of organic molecules owing to the fact of being highly parameterized. The next step was a re-optimization of the MM+ optimized structures by applying semi-empirical PM3 molecular modeling method. Obtained files were further used for calculations.

Descriptors were calculated using Dragon 5.5 (>1500 descriptors) [11]. The correlation coefficients for all pair of descriptor variables used in the models were evaluated to identify highly correlated descriptors in order to detect redundancy in the data set. Hence, descriptors with constant variables and near-constant variables were excluded from the further consideration ($r \geq 0.95$) [11,12].

The genetic algorithm (GA) and multiple linear regression analysis (MLRA) were used to select the descriptors and to generate the correlation models that relate the structural features to the cell growth percent of different cancer cell lines. The combination of the GA–MLRA technique was applied to obtain the best QSAR models using the QSARINS 2.2. The latter program splitted compounds data as following: random selection of 20% of compounds for prediction set and 80% for training set. For each obtained model, such random selection was different [13,14].

Calculation of QSAR-models was conducted separately for each of 60 cell lines. Growth percent according to the NCI protocol was not converted to any other value; it was used in original version to built models. Some cell lines were given the value of −999, which means, that they were not tested.

The amount of generation algorithm setup was set until four descriptors, and generation per size was established to the value of 3000, and the division into training and test sets was performed automatically at a ratio of 80 to 20% relatively. Models, which showed statistical significance according to the parameters at a higher level ($r^2 \geq 0.5$), were selected for a more thorough rendering. For these lines, the following options were given: the amount of generation algorithm setup was set until seven descriptors, and generation per size was established to the value of 10,000. Substances were spited into training and test sets, and the division was made such as to establish equal distribution of substances of high and moderate percentage of inhibition of cell growth.

Docking, scoring, and visual inspection of synthesized substances into the enzymes binding sites. Flexible molecular docking was carried out using the software package OPENEYE by the reported earlier procedure [3,15–17].

Antimicrobial and antifungal activity. Agar-diffusion method was used for determination of the preliminary *in vitro* antibacterial and antifungal activity in Zaporizhzhya Regional Hospital Bacterial Laboratory against Gram positive bacteria (*S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, hospital *Streptococcus* spp.) and Gram negative bacteria (*Enterobacter aerogenes* 12, *Cronobacter sakazakii* ATCC BAA-894, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *Klebsiella pneumonia* 68) according to the earlier reported procedure [3].

RESULTS AND DISCUSSION

Chemistry. The following amides (**1.1–1.5**; **2.1–2.13**; **3.1–3.8**) were synthesized by classical methods, namely

alkylation of the potassium salt **1** by halogen-acylamides (A–C) refluxing in propan-2-ol-water solution (3:1). Reaction went smoothly with formation of the following amides (Fig. 2).

Spectral data (FT-IR, LC-MS, ^{1}H NMR) and elemental analysis confirmed the structure of synthesized substances and corresponded to the previous [1,2,4]triazolo[1,5-*c*] quinazoline derivatives data [2–5].

In LC-MS spectra, all compounds had a high-intensive peaks of $[M+H]^{+\cdot}$ in accordance with their molecular weight.

In IR spectra, the synthesized amides had vibrations of the carbonyl band at the 1680–1635 cm^{-1} for the secondary amides **1a–1l**, which shifted to the 1680–1630 cm^{-1} for aryl substituted amides **3a–3k**. Stretching NH was detected as multiplet at the 3307–3324 cm^{-1}. Intensive bands of $\nu_{C\text{-}N}$ and $\delta_{N\text{-}H}$ appeared at the 1556–1517 cm^{-1} – for aliphatic amides and at the 1556–1516 cm^{-1} – for aryl amides. The intense vibrations of CCl bond of substances **1a–1c**, **3a** were registered in the 746–717 cm^{-1} region, CBr for **1d**, **1e**, **3b**, **3c** at the 658–623 cm^{-1}, CF for **1f–1h** and **3d**, **3e** at the 1101–1005 cm^{-1}. Vibrations of CF_3-Ar were presented as the middle and strong intensive peaks at the 1323–1310 cm^{-1} and the 1185–1178 cm^{-1}.

In ^{1}H-NMR spectra, the [1,2,4]triazolo[1,5-*c*] quinazoline system of substances **1a–1l** was characterized by the following signals: singlet of H-5 at the 9.37–9.34 ppm, doublets H-10 at the 8.47–8.37 ppm and H-7 at the 8.03–8.02 ppm, triplets H-8 at the 7.90–7.88 ppm and H-9 at the 7.78–7.77 ppm correspondingly to the previous data [5,9]. The signals of heterocyclic **2a–2c** amides shifted a little bit to the low field: singlet H-5 to the 9.38–9.36 ppm, doublets H-10 to the 8.42–8.39 ppm, and H-7 to the 8.02–8.01 ppm, triplets H-8 to the 7.88–7.87 ppm, and H-9 to the 7.77–7.76 ppm. The signals of aromatic **3a–3k** amides were the following: singlet H-5 (9.42–9.37 ppm), doublets H-10 (8.45–8.42 ppm) and H-7 (8.03–8.01 ppm), triplets H-8 (7.90–7.87 ppm), and H-9 (7.79–7.77 ppm). One proton broadened singlet of NH group of benzyl amides **1a–1l** was fixed in the aromatic part of the spectrum at the 8.73–8.56 ppm, for heterocyclic amides **2a–2c** appeared at the 12.81–12.51 ppm and at the 11.95–9.53 ppm – for phenyl amides **3a–k**. Considering two proton singlet of SCH_2-group, it resonated at the 4.09–4.02 ppm for **1a–1l**, 4.37–4.34 ppm for **2a–2c**, and at the 4.28–4.15 ppm for **3a–3k**. For substances **3i** and **3j**, the spectra were characterized by broadened one protoned singlet of COOH group at the 13.32 and the 12.61 ppm. The phenyl protons of amides were found in the aromatic part of the spectrum, alkyl – in the strong field with appropriate multiplicity.

Antimicrobial activity

In vitro studies. All synthesized substances were investigated *in vitro* by agar-diffusion method in concentration of 5 mg/disk against Gram positive bacteria *S. aureus* and *E. faecalis*; Gram negative bacteria *E. aerogenes*, *P. aeruginosa*, *C. sakazakii*, *E. coli* and *K. pneumonia*; antifungal properties against *C. albicans*, using inhibition zone diameter (mm) as a measure for the antimicrobial activity (Table 1).

Unfortunately, *E. aerogenes*, *P. aeruginosa*, *C. sakazakii*, *K. pneumonia*, and *C. albicans* were insensitive to all synthesized compounds, and substances **1b–1d**, **1f**, **1h–1k**, **2c**, **3a–3d**, and **3f–3i** had no activity against all investigated microbial strains.

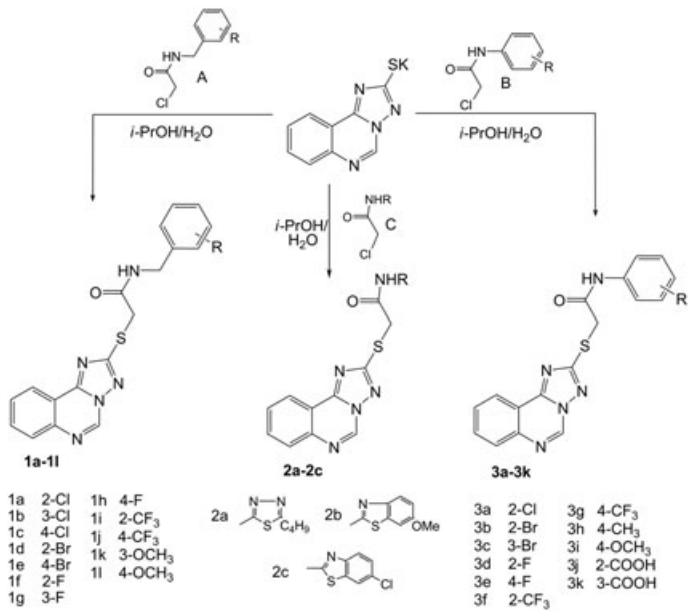

Figure 2. Synthesis of 2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylsulfanyl)acetamides.

Table 1

The inhibitory zones of the synthesized compounds, mm.

Compd.[a]	Conc. mg/disk	*E. coli*	*S. aureus*	*E. faecalis*
1a	5	6[b]	6	**7**[c]
1e	5	6	**10**	6
1g	5	6	**8**	6
1l	5	6	6	**8**
2a	5	6	**9**	6
2b	5	6	6	**7**
3e	5	6	**15**	**13**
3j	5	6	**10**	6
3k	5	**15**	6	6
Oxacillin	0.1	0	21	0
Vancomycin	0.1	16	18	0
Nystatin	0.1	0	11	15

[a]Substances **1b–1d**, **1f**, **1h–1k**, **2c**, **3a–3d**, **3f–3i** had no activity.
[b]Disk diameter.
[c]Bold values mean active compounds.
E. coli, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *E. faecalis*, *Enterococcus faecalis*.

Only nine substances had from light to moderate antibacterial activities. The best one was shown by *N*-(4-fluorophenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio) acetamide **3e**, which inhibited growth of *S. aureus* at the 15 mm and *E. faecalis* at the 13 mm. Interestingly, that compound **3d** with practically the same structure had no effect.

Besides, *E. coli* was sensitive only to 3-{[([1,2,4]triazolo [1,5-*c*]quinazolin-2-ylthio)acetyl]amino}benzoic acid **3k**, despite practically the same structure of **3j**. The light effect on the *S. aureus* growth was made by **1e**, **1g**, **2a**, and **3j**, and against *E. faecalis* by **1a** and **1l**, but still in lower, than the references concentration.

Comparing the obtained antimicrobial results with previous reported ones, it was found that introduction of hetaryl, 4-fluorophenyl and benzoic acid residues lead to the antimicrobial activity manifestation against *S. aureus* and *E. faecalis*.

***In silico* studies**

Molecular docking to E. faecalis dihydrofolate reductase. To predict the possible antibacterial mechanism of substances as well as to compare *in silico* results with *in vitro*, it was decided to conduct flexible molecular docking of the synthesized compounds [16].

Dihydrofolate reductase (DHFR) is a classic drug target, because it promotes the NADPH-dependent reduction of 7,8-dihydrofolate to yield 5,6,7,8-tetrahydrofolate, which is involved in the biosynthesis of purines, thymidylate, and several amino acids in microbial cells [18]. Moreover, investigation of hydrophobic heterocyclic dihydrophthalazines series, which were designed from antifolate drug trimethoprim, the propyl and trifluoropropyl substituents had an important role in protein stability during catalytic cycling, due to flexibility – an important determinant to fit into the inhibitor, thereby allowing it to take advantage of any available subpockets of the binding site [19].

The software package «OpenEye» was used for studies, including utilities: Fred Receptor 2.2.5, Vida 4.1.1, Flipper, Babel 3, Omega 2.4.3 and Fred 2.2.5 [15].

The crystal structure of the enzyme *E. faecalis* DHFR (4M7U.pdb) was obtained from the protein data bank, and Trimetoprim was used as reference [17,19].

The obtained scoring functions (Shapegauss, PLP, Chemgauss2, Chemgauss3, Chemscore, OEChemscore, Screenscore, CGO, CGT, Zapbind, Consensus Score) indicated the best possibility of the matching into the ligand-protein complex (Table S2).

Practically, all substances had better consensus scores, than Trimetoprim, except of **3g**. All heterocyclic amides, namely, substance **2b** had practically five times better affinity, than the reference, and **2a** with **2c** had a little bit worth result, still high enough.

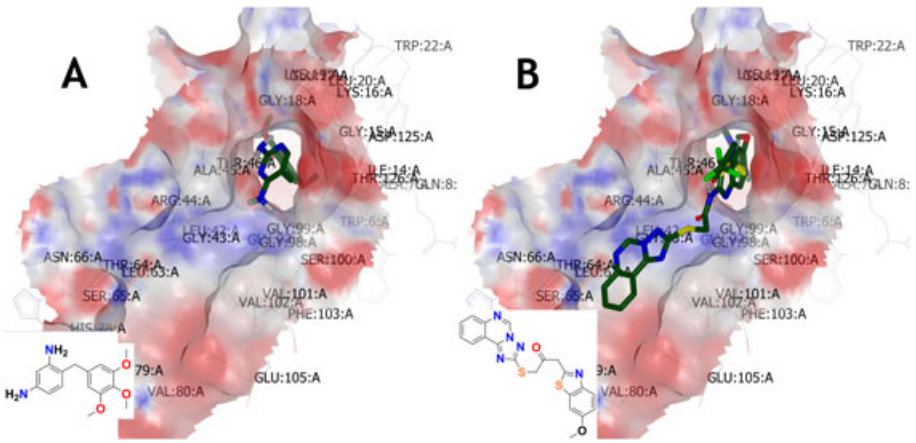

Figure 3. Visual representation of a receptor–ligand interaction: active site of *Enterococcus faecalis* dihydrofolate reductase (4M7U.pdb) with Trimetoprim (A) and *N*-(6-methoxy-1,3-benzothiazol-2-yl)-2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetamide **2b** (B), which has the highest Consensus Score. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The visual inspection demonstrated that substituents at the second position were flexible enough to be rotated by the Sulfur bond and intercorporated in the same enzyme pocket as Trimetoprim (Fig. 3), although no hydrogen bonds were formed.

The good impact for the affinity was made by 3-OCH_3, 2-CF_3, 2-F, 2-Cl radicals in phenyl residue in series **1** and **3**. Interestingly, that the weakest substance **3g**, that differed from **1j** just by the absence of CH_2 fragment had the worst Consensus Score in 116 points.

Molecular docking to UDP-N-acetylglucosamine enolpyruvyl transferase. The essential enzyme UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA) that was involved in the biosynthesis of peptidoglycan of cell wall was also chosen to be investigated for molecular docking. Because Mur enzymes are absent in humans and have no human homologues, its inhibition represents a promising point of study [20,21].

Thus, $MyrA_{ec}$ from *E. cloacae* (1Q3G.pdb) was used for investigation [20]. The T6361 (2-(*N*-methyl-5-(naphthalen-1-ylsulfonyloxy)-2-(naphthalene-1-sulfon-amido) benzamido)-succinic acid, which was proven to inhibit MurA and MurZ of *E. faecalis*, was used as the reference [21].

No substances suppressed the T6361 by affinity into the $MyrA_{ec}$ binding site (Table S3). Visual inspection also had shown the sterical restrictions of the structures to fit into its pocket (Fig. 4).

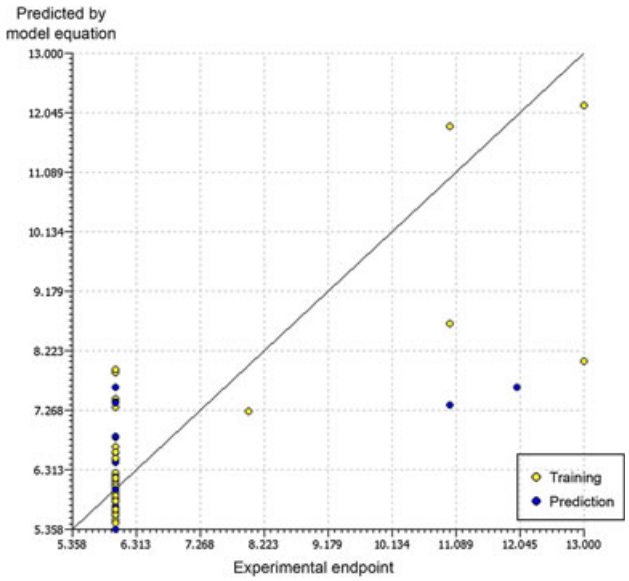

Figure 5. Correlation graph of predicted versus experimental growth percentage for model equation against *Streptococcus* sp. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Hitherto, analyzing substances' structure with the best consensus scores (**3k**, **1k,** and **1b)**, among the most important factors to fit into enzyme were the following: the third

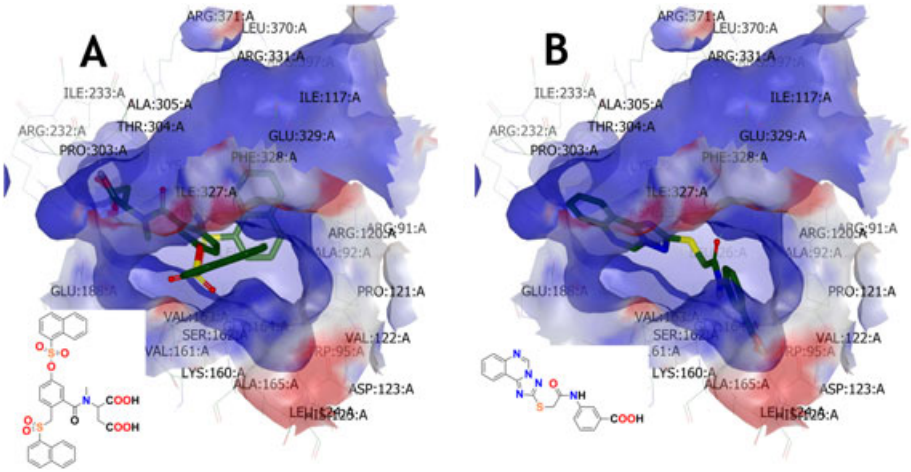

Figure 4. Visual representation of a receptor–ligand interaction: active site of *Enterobacter cloacae* $MyrA_{ec}$ (1Q3G.pdb) with T6361 (A) and 3-{[([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetyl]amino}-benzoic acid **3k** (B), which has the highest consensus score. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 2

The best QSAR-model's statistical characteristics.

Training set*						Prediction set		
n	r^2	RMSE	*s*	*F*	Q^2_{LOO}	*n*	r^2	RMSE
59	0.6489	0.9145	0.9472	33.8846	0.4856	14	0.4956	1.7155

*n, number of investigated compounds in set, r^2, squared regression coefficient, RMSE, root mean square error, s, standard error, F, variance ratio, Fisher coefficient, Q^2_{LOO}, weighted correlation coefficient by leave-one out method.

position of the substituent in the phenyl ring, the possibility of the phenyl ring rotation, due to $NHCH_2$ fragment presence for compounds **1d** and **1i,** and absence of the F, Br, CH_3 and CF_3 radicals in phenyl ring.

Hence, according to the docking results, inhibition of the $MyrA_e$ was doubtable to be the possible way of activity, although the most active substance **3k** effected the growth of *E. coli*, but inhibition of DHFR could be suggested as one of the possible antimicrobial activity mechanism, although according to *in vitro* results series **2** had poor antimicrobial activity.

Yet, the presence of the proposed mechanisms should be proven by subsequent enzymatic studies, which we still had no opportunity to do.

Quantitative structure–activity relationship studies. Considering the low antimicrobial properties of the synthesized compounds, it was decided to conduct QSAR studies based on the already reported antimicrobial data of the 2-thiosubstituted [1,2,4] triazolo[1,5-*c*]quinazolines with consequent prediction of the antimicrobial potential for all their synthesized 2-thio series (Table S4, Mol files) [2–5].

The program QSARINS 2.2 calculated the best regression using descriptors obtained by Dragon 5.5 [13,14]. Because of their very high quantity, GA and MLRA were used.

Hence, all bacteria and fungi strains were analyzed, and substances descriptors were found, but only one equation against *Streptococcus* sp. had squared regression coefficient (r^2) at least 0.6489, and weighted correlation coefficient by leave-one out method (Q^2_{LOO}) was 0.4856, due to analyzed variance with low exhibited *in vitro* antibacterial activity (Table 2, Fig. 5).

Upon these data, the simple transparent and realistic QSAR model was proposed to avoid errors arising from

Table 3

Experimental data of substances *in vitro* antimicrobial activity in comparison to predicted by QSAR-model, mm.

Cmpd.[a]	Status	*In vitro*	Predicted	Cmpd	Status	*In vitro*	Predicted
LA-45	Prediction	6	5.36	MN-44	Prediction	6	6.07
MN-17	Training	6	5.47	MN-56	Training	6	6.11
LA-26	Training	6	5.49	MN-52	Training	6	6.13
LA-28	Training	6	5.51	LA-16	Training	6	6.14
LA-12	Prediction	6	5.59	LA-13	Training	6	6.16
MN-13	Training	6	5.61	MN-54	Training	6	6.16
LA-3	Training	6	5.63	MN-66	Training	6	6.18
LA-29	Training	6	5.66	MN-53	Training	6	6.20
MN-69	Training	6	5.68	LA-23	Training	6	6.22
MN-50	Training	6	5.74	LA-185	Training	6	6.24
MN-67	Prediction	6	5.74	LA-256	Training	6	6.28
MN-12	Training	6	5.80	LA-40	Prediction	6	6.43
LA-47	Training	6	5.82	LA-259	Training	6	6.47
MN-46	Training	6	5.82	LA-5	Training	6	6.51
MN-64	Prediction	6	5.82	LA-2	Training	6	6.59
MN-68	Training	6	5.82	LA-27	Training	6	6.61
LA-102	Training	6	5.84	LA-15	Training	6	6.68
LA-44	Training	6	5.84	LA-1	Training	6	6.84
LA-84	Training	6	5.84	LA-254	Prediction	6	6.85
LA-46	Training	6	5.86	LA-177	Training	8	7.26
LA-14	Training	6	5.90	LA-178	Training	6	7.32
MN-58	Prediction	6	5.90	LA-232	Prediction	11	7.36
MN-62	Training	6	5.90	LA-230	Prediction	6	7.40
MN-60	Training	6	5.93	LA-216	Training	6	7.42
LA-183	Training	6	5.95	LA-211	Training	6	7.45
MN-55	Training	6	5.95	LA-139	Prediction	12	7.65
MN-57	Training	6	5.95	LA-200	Prediction	6	7.65
MN-59	Training	6	5.95	LA-49	Prediction	6	7.65
MN-45	Training	6	5.97	LA-187	Training	6	7.88
MN-49	Training	6	6.05	MN-47	Training	6	5.97
MN-61	Training	6	6.05	MN-48	Training	6	5.97
LA-10	Training	6	5.99	LA-186	Training	13	8.07
MN-51	Training	6	5.99	LA-39	Training	11	8.66
LA-80	Training	6	6.01	LA-50	Training	11	11.84
MN-65	Prediction	6	6.01	LA-255	Training	13	12.16
LA-48	Training	6	7.93	-	-	-	-

[a]Original names of compounds were used not to be confused with each other, because of the same numbering in different articles (Supporting Information, mol files).

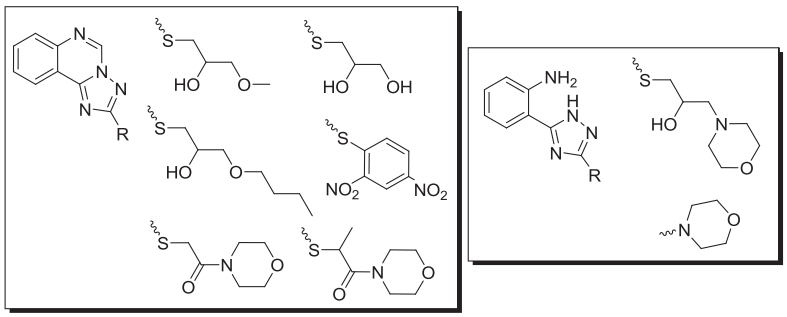

Figure 6. The most active compounds predicted *in silico* by QSAR model among 2-thiosubstituted [1,2,4]triazolo[1,5-*c*]quinazolines and their 2-(2-R-1*H*-1,2,4-triazol-5-yl)aniline analogues against *Streptococcus* species.

over-fitting of data: 19.1944*R3e++1.963*O-056 +4.4204*O-059+4.5322. So, the essential descriptors were R3e: weighted by atomic Sanderson electronegativities – R autocorrelation of lag 3, atom centered fragments: O-056 – alcohol and O-059 – Alk-O-Alk [12].

The prediction of the antimicrobial activity by the calculated QSAR model showed its moderate accuracy (Table 3).

Then, the calculated QSAR model was used to predict antimicrobial properties against *Streptococcus sp*. by other 270 reported and unreported 2-thio-substituted [1,2,4] triazolo[1,5-*c*]quinazolines (Table S5).

Only 12 substances had at least 10–12 mm of growth inhibition predicted. The best results were predicted for alcohols, 2,4-dinitrophenyl derivative and morpholino substituted amides of 2-thio-[1,2,4]triazolo[1,5-*c*] quinazoline carboxylic acids (Fig. 6, Table S6).

Moreover, nucleophilic degradation to 2-(1*H*-1,2,4-triazol-5-yl)anilines and direct introduction into [1,2,4] triazolo[1,5-*c*]quinazoline second position of the morpholine heterocycle also had positive impact for exhibiting the antimicrobial activity.

But considering the small amount and low predicted activity, future studies of the antimicrobial activity among 2-thio-[1,2,4]triazolo[1,5-*c*]quinazolines are not desirable without chemical modifications in the fifth position or benzene ring of quinazoline mojety.

CONCLUSIONS

Thus, despite the presence of the several antimicrobial pharmacophores in the structure of the *N*-(phenyl, benzyl, hetaryl)-2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetamides, it was proven by *in vitro* and *in silico* studies that synthesis of the amides series had no positive impact into promotion of their antimicrobial activity. Investigated compounds had considerable affinity predicted *in silico* to DHFR, still only nine substances inhibited growth of *E. faecalis*, *S. aureus*, or *E. coli*. Among them, *N*-(4-fluorophenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetamide **3e** and 3-{[([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)-acetyl] amino}benzoic acid **3k** were the strongest ones. The flexibility of thio substituents as well as the presence of the benzoic acid, hetaryl and 4-fluorophenyl fragments appeared to be the crucial factors for substances to be antimicrobials.

Thus, the reported results indicated that *in vitro* screening was a significant and important methodology as the starting point of drug candidates' search, even with good *in silico* molecular docking results [22]. Moreover, the best way of future modifications to manifest [1,2,4]triazolo[1,5-*c*]quinazolines' antibacterial activity will be hetaryl groups introduction directly into the 2nd position according to the QSAR prediction, and considering already reported positive results among potassium 2-hetaryl-[1,2,4]triazolo [1,5-*c*]quinazoline-5-thiolates, removing the thio group from the 2nd into the 5th position.

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