Arabian Journal of Chemistry (2016) 9, 792–805

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Potential of *N*-aryl(benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides as anticancer and antimicrobial agents

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Received 7 November 2013; accepted 4 September 2014
Available online 28 September 2014

KEYWORDS

Anticancer;
Antibacterial;
Antifungal;
Bioluminescence;
N-aryl(benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides

Abstract The death rate from cancer and infectious diseases is still very high, therefore research in this area is extremely important and promising as in medical, so in economic point of view. Thus, potassium salt of tetrazolo[1,5-*c*]quinazolin-5-thion was modified per alkylation by *N*-aryl(benzyl,heteryl)acetamides with proper confirmation of newly synthesized compounds' structures by FT-IR, LC–MS, ^{1}H NMR and elemental analysis data. The substances were tested for bioluminescence inhibition against *Photobacterium leiognathi* Sh1 (5–50 μg/mL) to check their cytotoxicity. Then they were screened for antibacterial and antifungal activities (100 μg) against *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*. It was found that compounds **1.1**, **1.5**, **1.10**, **1.31**, **1.33** possessed light activity against *K. pneumonia*. The US National Cancer Institute (NCI) has chosen 19 compounds and screened them for ability to inhibit in 10 μM concentration 60 different human tumor cell lines. The LOX IMVI cell line of melanoma appeared to be the most sensitive one, and *N*-(6-methylbenzo[*d*]thiazol-2-yl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamide (**1.31**) and *N*-(3-fluorobenzyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamide (**1.19**) exhibited high growth inhibition rate, and *N*-(6-methoxybenzo[*d*]thiazol-2-yl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamide (**1.32**) showed lethal antitumor activity against it. The latter compound **1.32** showed the best anticancer results, also inhibiting growth of leukemia SR cell line,

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.09.009

NCI-H460 of non-small cell lung cancer, KM12 of colon cancer and SF-295 of CNS cancer. The *in silico* molecular docking studies have predicted the affinity of the synthesized substances to the epidermal growth factor receptor (EGFR).

1. Introduction

Annually in the world about 13 million of new oncological cases are registered and more than 6.2 million deaths occur, according to WHO data. As you can see, death rate is very high. The situation with bacterial infections is no better. The WHO report of 2014 about antimicrobial resistance warns that resistance to common bacteria, viruses and fungi has reached alarming levels in many parts of the world, and a post-antibiotic era, in which common infections and minor injuries can kill, is a very real possibility for the 21st Century. Nevertheless, more and more people are surviving cancer, thanks to advances in treatment and screening tests that could predict or detect it. That is why anticancer and antimicrobial agent investigations are very important and always up-to-date. Our research group is dealing with substituted tetrazolo[1,5-*c*]quinazolines (Antypenko et al., 2013), [1,2,4]triazolo[1,5-*c*]quinazolines (Antipenko et al., 2009a,b) and [1,2,4]triazino[2,3-*c*]quinazolines (Nosulenko et al., 2014; Berest et al., 2011, 2012; Kovalenko et al., 2012), that were already investigated to have various biological activities: antibacterial, antifungal, anticancer, antiviral and antioxidant. The potent EGFR inhibitors – anticancer drugs such as Gefitinib, Erlotinib, Lapatinib, Vandetanib or Saracatinib (Cao et al., 2006; Carlomagno et al., 2002; Konecny et al., 2006; Rusnak et al., 2001) are quinazoline derivatives from chemical aspect. Thus, 2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)-1-(p-tolyl)ethanone showed lethal leukemia CCRF-CEM cell line growth inhibition (−49.78 growth percent), 3-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)propanoic acid to −44.08%, 3-methyl-2-(tetrazolo [1,5-c]quinazolin-5-ylthio)butanoic acid to −55.61%, and methyl (tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetate to −32.45% (Antypenko et al., 2013). Moreover, condensed tetrazoles: tetrazolo[1,5-*a*]quinoline-4-carbonitriles (Kategaonkar et al., 2010), dihydrotetrazoloquinazolines and dihydrotetrazoloquinazolinones (Ellis et al., 1980; Jantova et al., 2004; Mohammed et al., 1991; Myznikov et al., 2007; Sherif et al., 2009) revealed antimicrobial activity. Among all the chemical structure modifications and introductions of the different functional residues into the main skeleton, the acetamide synthesis was chosen, since it was found, that *N*-cyclohexyl-2-[5-(4-pyridyl)-4-(*p*-tolyl)-4*H*-1,2,4-triazol-3-ylsulfanyl]acetamide dihydrate had been synthesized and demonstrated good antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa and Klebsiella pneumonia*), antifungal (*Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes*) and antioxidant activities (Orek et al., 2012). Moreover, 4-phenyl-1-napthyl phenyl acetamide showed antifungal (*Aspergillus*, *Candida* species) activity (Saha et al. 2012); some 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides were strong antibacterial (*β-Hemolytic streptococcus* and *K. pneumonia*, *E. coli* and *Pseudomonas*) and antifungal (*A. flavus*, *Microsporum gypseum*, *Mucor*, *Rhizopus*) agents (Kanagarajan et al., 2010); and benzimidazolyl-1,3,4-oxadiazol-2-ylthio-*N*-phenyl(benzothiazolyl)acetamides possessed antibacterial (*S. aureus*, *Bacillus cereus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*, *Shigella flexneri*) and antitubercular (*Mycobacterium tuberculosis*) properties (Patel et al., 2012). Hence, the aim of this research is to unleash the potential of novel *N*-aryl (benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides as antibacterial, antifungal and anticancer agents.

2. Experimental

Melting points were determined in open capillary tubes in a «Stuart SMP30» apparatus and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL cube analyzer. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer using a module ATR eco ZnSe. ^1H NMR spectra (400 MHz) were recorded at a Varian-Mercury 400 and Bruker Avance DRX-500 spectrometers with $SiMe_4$ as internal standard in DMSO-d_6 solution. LC–MS were recorded using chromatography/mass spectrometric system which consists of high-performed liquid chromatograph «Agilent 1100 Series» equipped with diode-matrix and mass-selective detector «Agilent LC/MSD SL» (atmospheric pressure chemical ionization – APCI).

2.1. General procedure for the synthesis of N-aryl(benzyl, heteryl)-2-(tetrazolo[1,5-c]quinazoline-5-ylthio)acetamides

Method A: *N*,*N*-Carbonyldiimidazole (0.71 g, 4.4 mmol) was added to a solution of 2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio) acetic acid (4.4 mmol) in 10 ml of anhydrous 1,4-dioxane and heated at the water bath at 60–80 °C for 1 h with calcium chloride tube. Then the proper *N*-aryl(benzyl,heteryl)-amine (4.4 mmol) was added to the resulting mixture and refluxed for 1 h with stirring. The mixture was poured into the water, neutralized to pH 6–7 by NaOH. The precipitate was filtered, dried and recrystallized from the mixture of 2-propanol:water (2:1).

Method B: *N*-aryl(benzyl,heteryl)-2-chloroacetamide (4.4 mmol) was added to a suspension of tetrazolo[1,5-C]quinazoline-5-thione potassium salt (4.4 mol) in 20 ml of 2-propanol and refluxed for 2 h. The mixture was cooled and poured into the water. The precipitate was filtered, dried and recrystallized from mixture of 2-propanol:water (2:1).

*2.1.1. N-(2-Methoxyphenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.1**)*

Yield = 85%; mp = 170–172 °C; ^1H NMR: δ (ppm): 9.56 (s, 1H, NH), 8.55 (d, J = 7.81 Hz, 1H, H-10), 8.05 (t, J = 7.51 Hz, 2H, H-7, Ph-6), 8.00 (t, J = 7.67 Hz, 1H, H-9), 7.82 (t, J = 7.36 Hz, 1H, H-8), 7.03 (t, J = 7.51 Hz, 1H,

Ph-4), 6.97 (d, J = 7.98 Hz, 1H, Ph-3), 6.87 (t, J = 7.35 Hz, 1H, Ph-5), 4.51 (s, 2H, SCH_2), 3.81 (s, 3H, OCH_3). IR (cm^{-1}): 3343, 2918, 2848, 1676, 1619, 1587, 1536, 1488, 1455, 1435, 1390, 1327, 1291, 1277, 1248, 1214, 1173, 1144, 1124, 1109, 1051, 1037, 1020, 983, 969, 930, 898, 875, 783, 771, 741, 714, 677, 669, 648. LC–MS: m/z = 367 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{14}N_6O_2S$: C, 55.73; H, 3.85; N, 22.94; S, 8.75. Found: C, 55.71; H, 3.89; N, 22.90; S, 8.79.

*2.1.2. N-(3-Methoxyphenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.2**)*

Yield = 99%; mp = 168–170 °C; ^1H NMR: δ (ppm) 10.40 (s, 1H, NH), 8.53 (d, J = 7.66 Hz, 1H, H-10), 8.00–7.93 (m, 2H, H-7,9), 7.79 (t, J = 7.08 Hz, 1H, H-8), 7.33 (s, 1H, Ph-2), 7.16 (dd, J = 18.72, 7.83 Hz, 2H, Ph-5,6), 6.60 (d, J = 6.67 Hz, 1H, Ph-4), 4.46 (s, 2H, SCH_2), 3.75 (s, 3H, OCH_3). IR (cm^{-1}): 3263, 3199, 3118, 3058, 3023, 2961, 2919, 2866, 2849, 2849, 2838, 1681, 1621, 1587, 1540, 1489, 1478, 1451, 1426, 1386, 1325, 1301, 1280, 1260, 1202, 1179, 1164, 1145, 1104, 1080, 1050, 1036, 973, 956, 897, 885, 872, 861, 807, 769, 750, 713, 687, 637, 616. LC–MS: m/z = 367 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{14}N_6O_2S$: C, 55.73; H, 3.85; N, 22.94; S, 8.75. Found: C, 55.79; H, 3.81; N, 22.97; S, 8.75.

*2.1.3. N-(4-Methoxyphenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.3**)*

Yield = 98%; mp = 176–178 °C; ^1H NMR: δ (ppm): 10.24 (s, 1H, NH), 8.54 (d, J = 7.5 Hz, 1H, H-10), 8.01–7.95 (m, 2H, H-7,9), 7.80 (t, J = 6.7 Hz, 1H, H-8), 7.52 (d, J = 7.4 Hz, 2H, Ph-2,6), 6.83 (d, J = 7.1 Hz, 2H, Ph-3,5), 4.43 (s, 2H, SCH_2), 3.75 (s, 3H, OCH_3). IR (cm^{-1}): 3325, 3256, 3198, 3059, 3005, 2961, 2919, 2851, 1676, 1657, 1619, 1590, 1537, 1511, 1493, 1475, 1462, 1452, 1439, 1414, 1382, 1325, 1288, 1276, 1248, 1237, 1194, 1159, 1108, 1036, 1027, 984, 970, 872, 827, 808, 793, 770, 715, 688, 668, 646, 614, 614. LC–MS: m/z = 367 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{14}N_6O_2S$: C, 55.73; H, 3.85; N, 22.94; S, 8.75. Found: C, 55.71; H, 3.89; N, 22.90; S, 8.79.

*2.1.4. N-(2-Fluorophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.4**)*

Yield = 82%; mp = 196–198 °C; ^1H NMR: δ (ppm) 10.19 (s, 1H, NH), 8.55 (d, J = 7.61 Hz, 1H, H-10), 8.00 (dd, J = 12.21, 6.92 Hz, 3H, H-7,9, Ph-6), 7.82 (t, J = 7.06 Hz, 1H, H-8), 7.22–7.07 (m, 3H, Ph-3,4,5), 4.53 (s, 2H, SCH_2). IR (cm^{-1}): 3323, 3258, 2998, 2920, 2850, 1660, 1619, 1590, 1538, 1495, 1475, 1453, 1405, 1382, 1323, 1276, 1258, 1194, 1160, 1137, 1103, 1036, 980, 969, 942, 905, 872, 855, 836, 803, 766, 754, 715, 688, 668, 642, 616. LC–MS: m/z = 355 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}FN_6OS$: C, 54.23; H, 3.13; N, 23.72; S, 9.05. Found: C, 54.26; H, 3.12; N, 23.79; S, 9.01.

*2.1.5. N-(3-Fluorophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.5**)*

Yield = 83%; mp = 212–214 °C; ^1H NMR: δ (ppm) 10.65 (s, 1H, NH), 8.52 (d, J = 7.21 Hz, 1H, H-10), 8.01–7.92 (m, 2H, H-7,9), 7.78 (t, J = 7.53 Hz, 1H, H-8), 7.59 (d, J = 11.20 Hz, 1H, Ph-6), 7.40–7.23 (m, 2H, Ph-4,5), 6.79 (s, 1H, Ph-2), 4.47 (s, 2H, SCH_2). IR (cm^{-1}): 3274, 2919, 2850, 1734, 1682, 1636, 1615, 1587, 1540, 1506, 1489, 1474, 1443, 1374, 1323, 1270, 1254, 1185, 1162, 1147, 1131, 1107, 1041, 1022, 1001, 985, 964, 919, 895, 867, 809, 770, 711, 684, 651, 638. LC–MS: m/z = 355 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}FN_6OS$: C, 54.23; H, 3.13; N, 23.72; S, 9.05. Found: C, 54.19; H, 3.17; N, 23.69; S, 9.09.

*2.1.6. N-(4-Fluorophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.6**)*

Yield = 77%; mp = 182–184 °C; ^1H NMR: δ (ppm) 10.44 (s, 1H, NH), 8.55 (d, J = 7.65 Hz, 1H, H-10), 8.02–7.94 (m, 2H, H-7,9), 7.80 (t, J = 7.06 Hz, 1H, H-8), 7.68–7.59 (m, 2H, Ph-2,6), 7.05 (t, J = 8.00 Hz, 2H, Ph-3,5), 4.45 (s, 2H, SCH_2). IR (cm^{-1}): 3353, 3065, 2921, 2850, 1732, 1684, 1673, 1641, 1618, 1587, 1542, 1511, 1495, 1479, 1454, 1408, 1384, 1329, 1281, 1254, 1228, 1217, 1160, 1109, 1043, 976, 891, 882, 870, 832, 810, 786, 770, 715, 686, 668, 636, 616. LC–MS: m/z = 355 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}FN_6OS$: C, 54.23; H, 3.13; N, 23.72; S, 9.05. Found: C, 54.27; H, 3.10; N, 23.75; S, 9.02.

*2.1.7. N-(2-Chlorophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.7**)*

Yield = 76%; mp = 180–182 °C; ^1H NMR: δ (ppm) 9.87 (s, 1H, NH), 8.55 (d, J = 6.37 Hz, 1H, H-10), 8.04 (d, J = 4.78 Hz, 1H, H-7), 7.98 (d, J = 5.37 Hz, 1H, H-9), 7.89–7.76 (m, 2H, H-8, Ph-6), 7.43 (d, J = 6.10 Hz, 1H, Ph-3), 7.29 (d, J = 5.03 Hz, 1H, Ph-5), 7.16 (d, J = 4.77 Hz, 1H, Ph-4), 4.55 (s, 2H, SCH_2). IR (cm^{-1}): 3745, 3609, 3244, 3004, 2929, 2852, 2177, 2089, 1690, 1654, 1619, 1588, 1564, 1534, 1489, 1474, 1451, 1441, 1382, 1320, 1275, 1238, 1212, 1159, 1139, 1108, 1058, 1036, 983, 969, 938, 907, 874, 840, 769, 753, 737, 717, 688, 679, 668, 642, 615. LC–MS: m/z = 371 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}ClN_6OS$: C, 51.82; H, 2.99; N, 22.66; S, 8.65. Found: C, 51.80; H, 3.04; N, 22.64; S, 8.67.

*2.1.8. N-(3-Chlorophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.8**)*

Yield = 75%; mp = 182–184 °C; ^1H NMR: δ (ppm) 10.58 (s, 1H, NH), 8.54 (d, J = 7.8 Hz, 1H, H-10), 7.97 (s, 2H, H-7,9), 7.80 (s, 2H, H-8, Ph-2), 7.50 (d, J = 7.9 Hz, 1H, Ph-6), 7.30 (t, J = 7.4 Hz, 1H, Ph-5), 7.05 (d, J = 7.6 Hz, 1H, Ph-4), 4.47 (s, 2H, SCH_2). IR (cm^{-1}): 3745, 3325, 3290, 3197, 3131, 3110, 3058, 2953, 2953, 2953, 2921, 2851, 1689, 1642, 1616, 1596, 1588, 1543, 1486, 1453, 1410, 1389, 1379, 1328, 1279, 1238, 1166, 1109, 1080, 1041, 996, 985, 974, 881, 787, 772, 713, 699, 684, 638, 604. LC–MS: m/z = 371 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}ClN_6OS$: C, 51.82; H, 2.99; N, 22.66; S, 8.65. Found: C, 51.78; H, 2.94; N, 22.67; S, 8.64.

*2.1.9. N-(4-Chlorophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.9**)*

Yield = 67%; mp = 202–206 °C; ^1H NMR: δ (ppm) 10.55 (s, 1H, NH), 8.55 (d, J = 7.7 Hz, 1H, H-10), 7.98 (s, 2H, H-7,9), 7.83–7.77 (m, 1H, H-8), 7.66 (d, J = 8.5 Hz, 2H, Ph-2,6), 7.29 (d, J = 8.4 Hz, 2H, Ph-3,5), 4.46 (s, 2H, SCH_2). IR (cm^{-1}): 3857, 3745, 3351, 3129, 3063, 2926, 2850, 1731, 1672, 1641, 1619, 1588, 1538, 1493, 1454, 1402, 1388, 1329, 1277, 1242, 1209, 1191, 1166, 1108, 1094, 1043, 1012, 976, 963, 891, 883, 865, 824, 787, 772, 733, 716, 687, 668, 646, 627, 616.

LC–MS: m/z = 371 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}ClN_6OS$: C, 51.82; H, 2.99; N, 22.66; S, 8.65. Found: C, 51.80; H, 3.01; N, 22.65; S, 8.69.

*2.1.10. N-(2-Bromophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.10**)*

Yield = 22%; mp = 156–160 °C; ^{1}H NMR: δ (ppm) 9.79 (s, 1H, NH), 8.56 (d, J = 7.47 Hz, 1H, H-10), 8.07 (d, J = 7.78 Hz, 1H, H-7), 8.00 (t, J = 6.72 Hz, 1H, H-9), 7.83 (t, J = 7.17 Hz, 1H, H-8), 7.75 (d, J = 6.91 Hz, 1H, Ph-6), 7.61 (d, J = 7.60 Hz, 1H, Ph-3), 7.35 (t, J = 7.67 Hz, 1H, Ph-5), 7.11 (d, J = 5.98 Hz, 1H, Ph-4), 4.53 (s, 2H, SCH_2). IR (cm^{-1}): 3747, 3244, 3003, 2919, 2850, 1654, 1632, 1620, 1589, 1563, 1535, 1491, 1475, 1452, 1438, 1381, 1324, 1276, 1237, 1199, 1161, 1139, 1108, 1037, 983, 970, 877, 877, 839, 769, 753, 732, 717, 688, 668, 659, 643, 615. LC–MS: m/z = 416 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}BrN_6OS$: C, 46.28; H, 2.67; N, 20.24; S, 7.72. Found: C, 46.24; H, 2.69; N, 20.28; S, 7.70.

*2.1.11. N-(3-Bromophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.11**)*

Yield = 72%; mp = 192–194 °C; ^{1}H NMR: δ (ppm) 10.57 (s, 1H, NH), 8.54 (d, J = 7.5 Hz, 1H, H-10), 7.96 (d, J = 12.7 Hz, 3H, H-7,9, Ph-2), 7.80 (s, 1H, H-8), 7.55 (d, J = 7.2 Hz, 1H, Ph-4), 7.30–7.12 (m, 2H, Ph-5,6), 4.46 (s, 2H, SCH_2). IR (cm^{-1}): 3311, 3243, 3174, 3131, 3079, 2954, 2919, 2850, 1681, 1659, 1650, 1642, 1632, 1621, 1588, 1564, 1555, 1536, 1503, 1490, 1470, 1453, 1410, 1385, 1317, 1278, 1263, 1238, 1161, 1108, 1081, 1037, 994, 974, 959, 886, 873, 863, 789, 770, 712, 680, 666, 640, 617. LC–MS: m/z = 417 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}BrN_6OS$: C, 46.28; H, 2.67; N, 20.24; S, 7.72. Found: C, 46.23; H, 2.66; N, 20.25; S, 7.71.

*2.1.12. N-(4-Bromophenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.12**)*

Yield = 83%; mp = 204–206 °C; ^{1}H NMR: δ (ppm) 10.53 (s, 1H, NH), 8.53 (d, J = 7.6 Hz, 1H, H-10), 7.96 (s, 2H, H-7,9), 7.79 (s, 1H, H-8), 7.60 (d, J = 7.5 Hz, 2H, Ph-2,6), 7.44 (d, J = 7.6 Hz, Ph-3,5), 4.46 (s, 2H, SCH_2). IR (cm^{-1}): 3746, 3310, 3059, 2927, 2852, 2165, 2114, 1785, 1729, 1689, 1673, 1641, 1632, 1620, 1589, 1573, 1536, 1488, 1453, 1387, 1326, 1276, 1242, 1192, 1164, 1108, 1071, 1042, 1008, 973, 962, 882, 821, 786, 771, 728, 715, 687, 668, 636, 614. LC–MS: m/z = 417 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}BrN_6OS$: C, 46.28; H, 2.67; N, 20.24; S, 7.72. Found: C, 46.21; H, 2.65; N, 20.27; S, 7.76.

*2.1.13. N-(3-(Trifluoromethyl)phenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.13**)*

Yield = 71%; mp = 206–208 °C; ^{1}H NMR: δ (ppm) 9.92 (s, 1H, NH), 8.57 (d, J = 7.82 Hz, 1H, H-10), 8.05–7.98 (m, 2H, H-7,9), 7.83 (t, J = 7.10 Hz, 1H, H-8), 7.70 (d, J = 7.64 Hz, 1H, Ph-6), 7.63 (t, J = 7.53 Hz, 1H), 7.57 (d, J = 7.79 Hz, 1H), 7.44 (d, J = 7.14 Hz, 1H), 4.51 (s, 2H, SCH_2). IR (cm^{-1}): 3248, 2997, 2926, 2850, 1657, 1619, 1589, 1537, 1489, 1451, 1381, 1316, 1273, 1238, 1176, 1161, 1106, 1056, 1037, 969, 904, 876, 854, 841, 761, 715, 665, 650, 604. LC–MS: m/z = 405 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{11}F_3N_6OS$: C, 50.49; H, 2.74; N, 20.78; S, 7.93. Found: C, 50.46; H, 2.78; N, 20.74; S, 7.97.

*2.1.14. N-(4-(Trifluoromethyl)phenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.14**)*

Yield = 95%; mp = 178–180 °C; ^{1}H NMR: δ (ppm) 10.72 (s, 1H, NH), 8.54 (d, J = 7.34 Hz, 1H, H-10), 8.07 (s, 1H, H-7), 7.96 (d, J = 7.01 Hz, 2H, H-9, Ph-2), 7.82 (dd, J = 12.66, 7.91 Hz, 2H, H-8, Ph-6), 7.51 (d, J = 7.19 Hz, 1H, Ph-3), 7.34 (d, J = 7.05 Hz, 1H, Ph-5), 4.49 (s, 2H, SCH_2). IR (cm^{-1}): 3325, 3296, 3244, 3160, 3115, 3050, 2979, 2923, 2851, 1697, 1617, 1586, 1556, 1494, 1454, 1382, 1336, 1327, 1280, 1238, 1184, 1164, 1132, 1111, 1100, 1076, 1043, 1001, 985, 974, 905, 887, 800, 787, 775, 699, 652, 652, 639, 604. LC–MS: m/z = 405 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{11}F_3N_6OS$: C, 50.49; H, 2.74; N, 20.78; S, 7.93. Found: C, 50.53; H, 2.71; N, 20.79; S, 7.91.

*2.1.15. N-(2-Methoxybenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.15**)*

Yield = 83%; mp = 186–188 °C; ^{1}H NMR: δ (ppm): 8.56 (d, J = 7.78 Hz, 1H, H-10), 8.52 (unsplit, 1H, NH), 8.01–7.94 (m, 2H, H-7,9), 7.81 (t, J = 3.94 Hz, 1H, H-8), 7.20 (d, J = 7.73 Hz, 1H, Ph-6), 7.16 (d, J = 7.73 Hz, 1H, Ph-3), 6.88 (d, J = 8.03 Hz, 1H, Ph-4), 6.76 (t, J = 7.30 Hz, 1H, Ph-5), 4.33 (d, J = 5.51 Hz, 2H, NHCH_2), 4.30 (s, 2H, SCH_2), 3.82 (s, 3H, OCH_3). IR (cm^{-1}): 3266, 3086, 2998, 2924, 2839, 1646, 1617, 1606, 1586, 1567, 1559, 1521, 1489, 1473, 1466, 1456, 1436, 1395, 1381, 1368, 1324, 1301, 1275, 1245, 1224, 1169, 1160, 1140, 1115, 1106, 1061, 1049, 1035, 1025, 983, 967, 900, 885, 877, 815, 784, 765, 743, 716, 643, 629. LC–MS: m/z = 381 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{16}N_6O_2S$: C, 56.83; H, 4.24; N, 22.09; S, 8.43. Found: C, 56.88; H, 4.20; N, 22.13; S, 8.41.

*2.1.16. N-(3-Methoxybenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.16**)*

Yield = 52%; mp = 174–176 °C; ^{1}H NMR: δ (ppm): 8.75 (unsplit, 1H, NH), 8.54 (d, J = 7.55 Hz, 1H, H-10), 7.98–7.91 (m, 2H, H-7,9), 7.81 (t, J = 5.68 Hz, 1H, H-8), 7.14 (t, J = 7.42 Hz, 1H, Ph-2), 6.87–6.79 (m, 2H, Ph-4,6), 6.73 (d, J = 7.54 Hz, 1H, PH-5), 4.33 (d, J = 5.15 Hz, 2H, NHCH_2), 4.29 (s, 2H, SCH_2), 3.71 (s, 3H, OCH_3). IR (cm^{-1}): 3736, 3275, 3084, 3001, 2960, 2921, 2851, 1724, 1690, 1650, 1615, 1587, 1556, 1519, 1490, 1474, 1453, 1432, 1383, 1325, 1288, 1275, 1263, 1226, 1150, 1108, 1072, 1036, 995, 979, 966, 897, 877, 851, 770, 741, 715, 689, 669, 639. Anal. Calcd. for $C_{18}H_{16}N_6O_2S$: C, 56.83; H, 4.24; N, 22.09; S, 8.43. Found: C, 56.79; H, 4.27; N, 22.03; S, 8.48.

*2.1.17. N-(4-Methoxybenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.17**)*

Yield = 76%; mp = 202–204 °C; ^{1}H NMR: δ (ppm): 8.66 (unsplit, 1H, NH), 8.55 (d, J = 7.41 Hz, 1H, H-10), 7.94 (d, J = 7.09 Hz, 1H, H-7), 7.90 (d, J = 8.00 Hz, 1H, H-9), 7.81 (t, J = 7.89 Hz, 1H, H-8), 7.18 (d, J = 8.33 Hz, 2H, Ph-2,6), 6.75 (d, J = 8.42 Hz, 2H, Ph-3,5), 4.28 (d, J = 5.74 Hz, 2H, NHCH_2), 4.25 (s, 2H, SCH_2), 3.74 (s, 3H, OCH_3). IR (cm^{-1}): 3281, 3063, 2989, 2919, 2841, 1744, 1713, 1644, 1614, 1587, 1550, 1513, 1495, 1472, 1463, 1442, 1398, 1378, 1363, 1324, 1306, 1273, 1247, 1220, 1185, 1167, 1137, 1106, 1066,

1029, 1007, 979, 969, 875, 843, 819, 785, 768, 716, 680, 641. LC–MS: m/z = 381 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{16}N_6O_2S$: C, 56.83; H, 4.24; N, 22.09; S, 8.43. Found: C, 56.87; H, 4.28; N, 22.04; S, 8.39.

*2.1.18. N-(2-Fluorobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.18**)*

Yield = 74%; mp = 184–186 °C; ^{1}H NMR: δ (ppm): 8.75 (unsplit, 1H, NH), 8.55 (d, J = 7.84 Hz, 1H, H-10), 7.98–7.93 (m, 2H, H-7,9), 7.81 (dd, J = 7.73, 2.91 Hz, 1H, H-8), 7.34 (t, J = 7.42 Hz, 1H, Ph-3), 7.23 (dd, J = 12.65, 6.19 Hz, 1H, Ph-4), 7.06 (d, J = 9.38 Hz, 1H, Ph-6), 7.00 (t, J = 7.72 Hz, 1H, Ph-5), 4.40 (d, J = 5.47 Hz, 2H, NHCH_2), 4.29 (s, 2H SCH_2). IR (cm^{-1}): 3376, 3330, 3066, 2926, 2847, 1674, 1615, 1585, 1538, 1488, 1453, 1424, 1380, 1361, 1318, 1275, 1254, 1226, 1201, 1173, 1164, 1108, 1041, 1030, 986, 974, 947, 897, 886, 830, 787, 775, 760, 715, 697, 680, 633. LC–MS: m/z = 369 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}FN_6OS$: C, 55.43; H, 3.56; N, 22.81; S, 8.70. Found: C, 55.47; H, 3.52; N, 22.86; S, 8.67.

*2.1.19. N-(3-Fluorobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.19**)*

Yield = 76%; mp = 192–196 °C; ^{1}H NMR: δ (ppm): 8.80 (unsplit, 1H, NH), 8.55 (d, J = 7.73 Hz, 1H, H-10), 7.98–7.92 (m, 2H, H-7,9), 7.84–7.77 (m, 1H, H-8), 7.27–7.20 (m, 1H, Ph-5), 7.08 (d, J = 7.55 Hz, 1H, Ph-4), 7.04 (d, J = 9.66 Hz, 1H, Ph-6), 6.92 (t, J = 7.77 Hz, 1H, Ph-2), 4.37 (d, J = 5.46 Hz, 2H, NHCH_2), 4.29 (s, 2H, SCH_2). IR (cm^{-1}): 3273, 3084, 2985, 2928, 2853, 1650, 1619, 1588, 1555, 1490, 1475, 1452, 1385, 1367, 1327, 1280, 1249, 1225, 1165, 1139, 1110, 1079, 1038, 1026, 983, 970, 930, 887, 875, 864, 767, 746, 713, 688, 645, 630. LC–MS: m/z = 369 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}FN_6OS$: C, 55.43; H, 3.56; N, 22.81; S, 8.70. Found: C, 55.46; H, 3.52; N, 22.85; S, 8.68.

*2.1.20. N-(4-Fluorobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.20**)*

Yield = 82%; mp = 192–194 °C; ^{1}H NMR: δ (ppm): 8.75 (unsplit, 1H, NH), 8.55 (d, J = 7.17 Hz, 1H, H-10), 8.02–7.86 (m, 2H, H-7,9), 7.80 (t, 1H, H-8), 7.28 (s, 2H, Ph-2,6), 6.95 (t, J = 7.95 Hz, 2H, Ph-3,5), 4.33 (d, 5.28 Hz, 2H, NHCH_2), 4.26 (s, 2H, SCH_2). IR (cm^{-1}): 3276, 3077, 2979, 2925, 2851, 1653, 1619, 1606, 1589, 1549, 1507, 1493, 1474, 1449, 1416, 1382, 1358, 1324, 1278, 1216, 1175, 1158, 1142, 1109, 1097, 1058, 1038, 1018, 981, 971, 894, 877, 851, 832, 814, 767, 715, 690, 645. LC–MS: m/z = 369 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}FN_6OS$: C, 55.43; H, 3.56; N, 22.81; S, 8.70. Found: C, 55.41; H, 3.59; N, 22.86; S, 8.66.

*2.1.21. N-(2-Chlorobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.21**)*

Yield = 77%; mp = 190–192 °C; ^{1}H NMR: δ (ppm): 8.76 (unsplit, 1H, NH), 8.56 (d, J = 7.85 Hz, 1H, H-10), 8.02–7.95 (m, 2H, H-7,9), 7.81 (t, J = 7.02 Hz, 1H, H-8), 7.36 (d, J = 7.66 Hz, 1H, Ph-3), 7.33 (d, J = 7.99 Hz, 1H, Ph-6), 7.20 (t, J = 7.29 Hz, 1H, Ph-5), 7.12 (t, J = 7.35 Hz, 1H, Ph-4), 4.43 (d, J = 5.65 Hz, 2H, NHCH_2), 4.33 (s, 2H, SCH_2). IR (cm^{-1}): 3367, 3324, 3063, 2920, 2849, 1675, 1615, 1585, 1540, 1493, 1472, 1452, 1442, 1418, 1379, 1359, 1316, 1277, 1255, 1208, 1186, 1170, 1159, 1144, 1132, 1108, 1071, 1039, 974, 897, 883, 804, 787, 773, 757, 714, 698, 679, 631. LC–MS: m/z = 385 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}ClN_6OS$: C, 53.06; H, 3.40; N, 21.84; S, 8.33. Found: C, 53.02; H, 3.45; N, 21.87; S, 8.30.

*2.1.22. N-(3-Chlorobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.22**)*

Yield = 99%; mp = 200–202 °C; ^{1}H NMR: δ (ppm): 8.81 (t, J = 5.03 Hz, 1H, NH), 8.55 (d, J = 7.85 Hz, 1H, H-10), 7.96–7.91 (m, 2H, H-7,9), 7.83–7.77 (m, 1H, H-8), 7.28 (s, 1H, Ph-2), 7.20 (d, J = 5.79 Hz, 3H, Ph-4,5,6), 4.36 (d, J = 5.88 Hz, 2H, NHCH_2), 4.28 (s, 2H, SCH_2). IR (cm^{-1}): 3353, 3269, 3051, 3051, 2921, 2851, 2104, 1650, 1618, 1600, 1586, 1555, 1491, 1474, 1452, 1430, 1385, 1360, 1326, 1279, 1228, 1163, 1139, 1109, 1077, 1038, 998, 983, 969, 842, 765, 713, 693, 676, 632. LC–MS: m/z = 385 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}ClN_6OS$: C, 53.06; H, 3.40; N, 21.84; S, 8.33. Found: C, 53.02; H, 3.45; N, 21.80; S, 8.37.

*2.1.23. N-(4-Chlorobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.23**)*

Yield = 73%; mp = 182–184 °C; ^{1}H NMR: δ (ppm): 8.78 (unsplit, 1H, NH), 8.56 (d, J = 7.53 Hz, 1H, H-10), 7.95 (d, J = 7.37 Hz, 1H, H-7), 7.89 (d, J = 8.00 Hz, 1H, H-9), 7.81 (t, J = 7.02 Hz, 1H, H-8), 7.26 (d, J = 7.64 Hz, 2H, Ph-3,5), 7.19 (d, J = 8.04 Hz, 2H, Ph-2,6), 4.33 (d, J = 5.47 Hz, 2H, NHCH_2), 4.26 (s, 2H, SCH_2). IR (cm^{-1}): 3285, 3065, 2989, 2927, 2853, 1639, 1617, 1588, 1547, 1492, 1474, 1453, 1410, 1397, 1378, 1322, 1299, 1274, 1248, 1220, 1170, 1138, 1107, 1091, 1067, 1035, 1019, 979, 969, 890, 876, 833, 818, 807, 784, 766, 716, 688, 642. LC–MS: m/z = 385 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}ClN_6OS$: C, 53.06; H, 3.40; N, 21.84; S, 8.33. Found: C, 53.03; H, 3.47; N, 21.81; S, 8.37.

*2.1.24. N-(2-Bromobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.24**)*

Yield = 84%; mp = 190–192 °C; ^{1}H NMR: δ (ppm): 8.78 (unsplit, 1H, NH), 8.57 (d, J = 7.04 Hz, 1H, H-10), 8.05–7.94 (m, 2H, H-7,9), 7.82 (t, J = 7.04 Hz, 1H, H-8), 7.51 (d, J = 7.16 Hz, 1H, Ph-3), 7.35 (d, J = 6.17 Hz, 1H, Ph-6), 7.20–7.09 (m, 2H, Ph-4,5), 4.38 (d, J = 5.64 Hz, 2H, NHCH_2), 4.33 (s, 2H, SCH_2). IR (cm^{-1}): 3327, 3274, 3220, 3049, 2921, 1732, 1674, 1651, 1615, 1585, 1540, 1504, 1491, 1469, 1451, 1438, 1416, 1379, 1320, 1277, 1254, 1210, 1187, 1169, 1144, 1107, 1071, 1040, 1023, 983, 972, 898, 881, 802, 787, 772, 756, 715, 697, 673, 631. LC–MS: m/z = 429 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}BrN_6OS$: C, 47.56; H, 3.05; N, 19.58; S, 7.47. Found: C, 47.52; H, 3.08; N, 19.56; S, 7.49.

*2.1.25. N-(3-Bromobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.25**)*

Yield = 73%; mp = 194–196 °C; ^{1}H NMR: δ (ppm): 8.80 (unsplit, 1H, NH), 8.55 (d, J = 7.66 Hz, 1H, H-10), 7.97–7.92 (m, 2H, H-7,9), 7.80 (t, J = 5.87 Hz, 1H, H-8), 7.43 (s, 1H, Ph-2), 7.33 (d, J = 7.54 Hz, 1H, Ph-4), 7.25 (d, J = 7.33 Hz, 1H, Ph-6), 7.15 (t, J = 7.57 Hz, 1H, Ph-5), 4.35 (d, J = 5.48 Hz, 2H, NHCH_2), 4.28 (s, 2H, SCH_2). IR (cm^{-1}): 3271, 3086, 3048, 2987, 2921, 2852, 1650, 1618, 1587, 1571, 1556, 1492, 1475, 1452, 1428, 1385, 1361, 1327, 1280, 1228, 1166, 1140, 1110, 1071, 1038, 1024, 998, 983, 970, 911, 911,

888, 877, 829, 829, 784, 766, 732, 714, 687, 667, 646, 632. LC–MS: m/z = 429 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}BrN_6OS$: C, 47.56; H, 3.05; N, 19.58; S, 7.47. Found: C, 47.59; H, 3.08; N, 19.54; S, 7.44.

*2.1.26. N-(4-Bromobenzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.26**)*

Yield = 66%; mp = 168–170 °C; ^{1}H NMR: δ (ppm): 8.78 (unsplit, 1H, NH), 8.55 (d, J = 7.61 Hz, 1H, H-10), 7.98 (d, J = 7.54 Hz, 1H, H-7), 7.88 (d, J = 7.92 Hz, 1H, H-9), 7.80 (t, J = 7.23 Hz, 1H, H-8), 7.33 (d, J = 8.01 Hz, 2H, Ph-3,5), 7.21 (d, J = 7.69 Hz, 2H, Ph-2,6), 4.32 (d, J = 5.28 Hz, 2H, NHCH_2), 4.26 (s, 2H, SCH_2). IR (cm^{-1}): 3275, 3079, 2985, 2935, 1722, 1690, 1650, 1619, 1588, 1553, 1535, 1489, 1475, 1453, 1433, 1418, 1377, 1349, 1326, 1278, 1228, 1157, 1108, 1071, 1038, 1012, 983, 969, 892, 827, 792, 765, 737, 715, 688, 668, 647, 617. LC–MS: m/z = 429 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}BrN_6OS$: C, 47.56; H, 3.05; N, 19.58; S, 7.47. Found: C, 47.54; H, 3.09; N, 19.54; S, 7.44.

*2.1.27. N-(2-(Trifluoromethyl)benzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.27**)*

Yield = 80%; mp = 180–182 °C; ^{1}H NMR: δ (ppm): 8.82 (unsplit, 1H, NH), 8.57 (d, J = 7.72 Hz, 1H, H-10), 8.04–7.96 (m, 2H, H-7,9), 7.82 (t, J = 7.28 Hz, 1H, H-8), 7.63 (d, J = 6.71 Hz, 1H, Ph-3), 7.54 (d, J = 7.00 Hz, 1H, Ph-6), 7.39 (dd, J = 12.30, 6.02 Hz, 2H, Ph-4,5), 4.55 (d, J = 4.93 Hz, 2H, NHCH_2), 4.34 (s, 2H, SCH_2). IR (cm^{-1}): 3272, 3082, 3003, 2922, 2851, 1731, 1653, 1621, 1589, 1556, 1493, 1477, 1454, 1434, 1384, 1313, 1274, 1230, 1212, 1195, 1172, 1163, 1118, 1096, 1072, 1058, 1037, 968, 900, 804, 785, 766, 731, 714, 688, 656, 630. LC–MS: m/z = 419 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{13}F_3N_6OS$: C, 51.67; H, 3.13; N, 20.09; S, 7.66. Found: C, 51.65; H, 3.16; N, 20.07; S, 7.63.

*2.1.28. N-(4-(Trifluoromethyl)benzyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.28**)*

Yield = 66%; mp = 192–194 °C; ^{1}H NMR: δ (ppm): 8.86 (unsplit, 1H, NH), 8.56 (d, J = 7.98 Hz, 1H, H-10), 7.96–7.89 (m, 2H, H-7,9), 7.81 (t, J = 7.52 Hz, 1H, H-8), 7.47 (q, J = 7.73, 7.50 Hz, 4H, Ph-2,3,5,6), 4.43 (d, J = 5.35 Hz, 2H, NHCH_2), 4.29 (s, 2H, SCH_2). IR (cm^{-1}): 3278, 3080, 2985, 2922, 2852, 1750, 1650, 1620, 1588, 1553, 1493, 1475, 1454, 1431, 1385, 1327, 1280, 1230, 1156, 1108, 1069, 1038, 1021, 983, 969, 833, 815, 768, 754, 729, 715, 689, 668, 649. LC–MS: m/z = 419 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{13}F_3N_6OS$: C, 51.67; H, 3.13; N, 20.09; S, 7.66. Found: C, 51.69; H, 3.11; N, 20.13; S, 7.67.

*2.1.29. 2-(Tetrazolo[1,5-c]quinazolin-5-ylthio)-N-(thiazol-2-yl)acetamide (**1.29**)*

Yield = 97%; mp = 232–234 °C; 12.57 (s, 1H, NH), 8.54 (d, J = 3.8 Hz, 1H, H-10), 7.94 (s, 2H, H-7,9), 7.79 (s, 1H, H-8), 7.45 (s, 1H, NCH), 7.10 (s, 1H, SCH), 4.55 (s, 2H, SCH_2). IR (cm^{-1}): 3743, 3197, 3118, 3064, 3046, 2918, 2849, 2725, 1688, 1616, 1583, 1556, 1486, 1472, 1450, 1383, 1327, 1266, 1246, 1210, 1174, 1140, 1107, 1074, 1037, 971, 904, 879, 844, 820, 773, 724, 711, 682, 668, 637, 623. Anal. Calcd. for $C_{13}H_9N_7OS_2$: C, 45.47; H, 2.64; N, 28.55; S, 18.68. Found: C, 45.44; H, 2.68; N, 28.51; S, 18.71.

*2.1.30. 2-(Tetrazolo[1,5-c]quinazolin-5-ylthio)-N-(1,3,4-thiadiazol-2-yl)acetamide (**1.30**)*

Yield = 92%; mp = 242–244 °C; ^{1}H NMR: δ (ppm) 13.06 (s, 1H, NH), 9.09 (s, 1H, NCH), 8.51 (d, J = 7.6 Hz, 1H, H-10), 7.94 (s, 1H, H-7), 7.89–7.80 (m, 2H, H-8,9), 4.81 (s, 2H, SCH_2). IR (cm^{-1}): 3177, 3094, 3043, 2918, 2849, 2732, 1705, 1615, 1576, 1492, 1473, 1450, 1380, 1338, 1277, 1266, 1252, 1229, 1175, 1140, 1104, 1052, 1040, 974, 907, 892, 880, 822, 775, 714, 687, 639, 606. Anal. Calcd. for $C_{12}H_8N_8OS_2$: C, 41.85; H, 2.34; N, 32.54; S, 18.62. Found: C, 41.81; H, 2.37; N, 32.53; S, 18.67.

*2.1.31. N-(6-Methylbenzo[d]thiazol-2-yl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.31**)*

Yield = 51%; mp = 216–218 °C; ^{1}H NMR: δ (ppm): δ 12.69 (s, 1H, NH), 8.53 (d, J = 7.6 Hz, 1H, H-10), 7.91 (dd, J = 18.0, 7.7 Hz, 2H, H-7,9), 7.75 (t, J = 7.2 Hz, 1H, H-8), 7.61 (d, J = 6.6 Hz, 2H, Ph-4,7), 7.19 (d, J = 8.0 Hz, 1H, Ph-5), 4.57 (s, 2H, SCH_2), 2.45 (s, 3H, CH_3). IR (cm^{-1}): 3275, 3079, 2985, 2935, 1722, 1690, 1650, 1619, 1588, 1553, 1535, 1489, 1475, 1453, 1433, 1418, 1377, 1349, 1326, 1278, 1228, 1157, 1108, 1071, 1038, 1012, 983, 969, 892, 827, 792, 765, 737, 715, 688, 668, 647, 617. Anal. Calcd. for $C_{18}H_{13}N_7OS_2$: C, 53.06; H, 3.22; N, 24.06; S, 15.74. Found: C, 53.04; H, 3.26; N, 24.04; S, 15.78.

*2.1.32. N-(6-Methoxybenzo[d]thiazol-2-yl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.32**)*

Yield = 98%; mp = 236–238 °C; ^{1}H NMR: δ (ppm): 12.99–12.57 (s, 1H, NH), 8.51 (d, J = 7.76 Hz, 1H, H-10), 7.95–7.88 (m, 2H, H-7,9), 7.77 (t, J = 7.89 Hz, 1H, H-8), 7.63 (d, J = 8.69 Hz, 1H, OCH_3CCHCH), 7.38 (s, 1H, $CHCOCH_3$), 6.97 (d, J = 8.65 Hz, 1H, OCH_3CCHCH), 4.56 (s, 2H, SCH_2), 3.80 (d, J = 2.81 Hz, 3H, OCH_3). IR (cm^{-1}): 3353, 3259, 3211, 3024, 2938, 2838, 1694, 1681, 1643, 1610, 1589, 1557, 1471, 1454, 1434, 1378, 1332, 1315, 1282, 1257, 1225, 1190, 1164, 1113, 1058, 1027, 983, 966, 900, 828, 799, 770, 714, 691, 665, 635. MS: m/z = 424 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{13}N_7O_2S_2$: C, 51.05; H, 3.09; N, 23.15; S, 15.14. Found: C, 51.09; H, 3.03; N, 23.11; S, 15.18.

*2.1.33. N-(6-Chlorobenzo[d]thiazol-2-yl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamide (**1.33**)*

Yield = 89%; mp = 236–238 °C; ^{1}H NMR: δ (ppm): 12.92 (s, 1H, NH), 8.54 (d, J = 7.15 Hz, 1H, H-10), 7.98–7.88 (m, 3H, H-7,9, CHCl), 7.83–7.76 (m, 1H, H-8), 7.74 (d, J = 8.45 Hz, 1H, CHCHCl), 7.40 (d, J = 8.10 Hz, 1H, CHCHCl), 4.61 (s, 2H, SCH_2). IR (cm^{-1}): 3744, 3257, 3174, 3120, 3079, 3063, 2994, 2924, 2849, 2175, 2116, 1693, 1619, 1587, 1548, 1491, 1476, 1446, 1434, 1376, 1330, 1308, 1256, 1220, 1156, 1110, 1097, 1054, 1035, 981, 965, 874, 803, 767, 752, 715, 685, 667, 634. Anal. Calcd. for $C_{17}H_{10}ClN_7OS_2$: C, 47.72; H, 2.36; N, 22.91; S, 14.99. Found: C, 47.68; H, 2.34; N, 22.96; S, 15.03.

3. Biological assays

3.1. Bioluminescence inhibition test

The marine luminescent bacteria *Photobacterium leiognathi* strain Sh1, isolated from the Azov Sea Shrimp, were used

for the bioluminescence analysis. They were cultivated at the nutrient environment containing (g/L): pepton – 5, yeast extract – 1.5, meat extract – 1.5, sodium chloride – 30, pH 7.4. In acute action test (inhibiting luminescence) bacteria were diluted with 3% sodium chloride solution up to concentration 10^5 cell/mL. The 5–50 μg/mL of the studied substances suspended in DMSO was mixed with 1 mL of the diluted bacterial suspension. Vials were incubated for 10 min at 25 °C, then intensity of bioluminescence was measured in% relatively to control tests which were prepared without the studied compounds. In chronic action test (inhibiting growth and luminescence) growth environment was added to the eventual breeding 1:50 and was incubated for 16–18 h at 30 °C, whereupon intensity of bioluminescence was measured as well as in the previous method. Tetracycline was used as a reference. The bacterial luminescence was measured with Bioluminometer BLM-8801 («Science», Krasnoyarsk, Russia).

3.2. Antimicrobial and antifungal test

All the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity in the Zaporozhyae Regional Hospital Bacterial Laboratory against Gram positive bacteria (*S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram negative bacteria (*Enterobacter aerogenes 12*, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *K. pneumoniae* 68). They were also evaluated for their *in vitro* antifungal potential against *Candida albicans* ATCC 885653. The amount of microbial cells was 1.5×10^8 cfu/mL. Incubation period of bacteria was 24 h at 35 °C, yeast – 48–72 h at 28–30 °C. The agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Standard sterilized filter paper disks (6 mm diameter) impregnated with a solution of the test compound in DMSO (100 μg/disk) were placed on an agar (Müller–Hinton Broth (Oxoid)) plate seeded with the appropriate test organism in triplicates. DMSO alone was used as control at the same above-mentioned concentration. Ampicillin, Ceftazide, Amikacin, Gentamycin, Ceftriaxone and Nystatin were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zone diameters (IZD) of bacterial or fungal growth around the disks in mm.

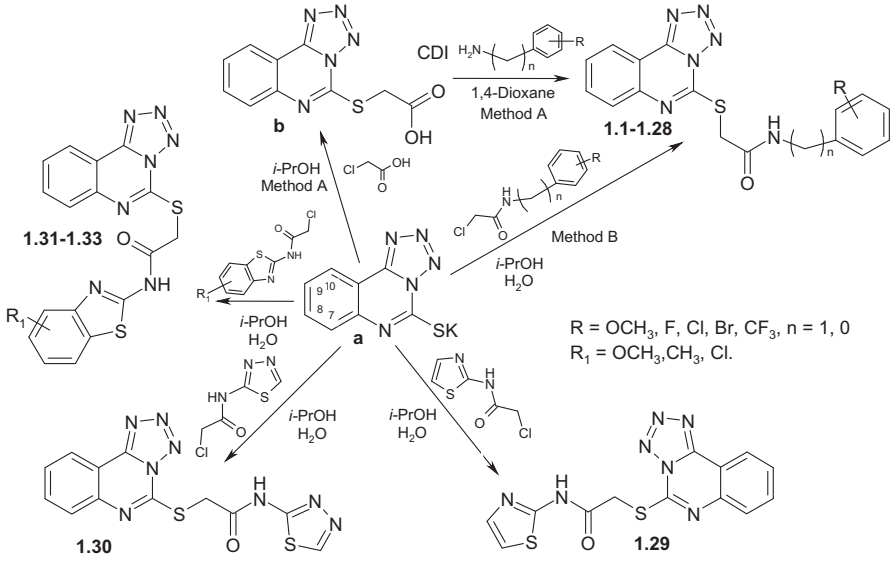
3.3. Antitumor activity

Primary anticancer assay was performed against human tumor cell line panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda (Boyd and Paull, 1995; Boyd, 1997). The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated in 96 well microtiter plates in 100 mL assay volume, at plating densities ranging from 5000 to 40000 cell/well. After cell inoculation, the microtiter plates were incubated at 37 °C, under an atmosphere of 5:95 CO_2:air (v/v) at 100% relative humidity, for 24 h prior to addition of drugs under assessment. Following drug addition (1 μM), the plates were incubated for an additional 48 h, under the same conditions. Sulforhodamine B (SRB) solution (100 μL, 0–4% w/v in 1% aq. acetic acid) was added to each well and plates were incubated for 10 min at room temperature. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

4. Results and discussion

4.1. Chemistry

Synthesis of the starting compound – potassium salt of tetrazolo[1,5-*c*]quinazolin-5-thion (**a**) was described in our previous work (Antypenko et al., 2013). The acetamides **1.1**–**1.33** were obtained by known methods: by aminolysis of activated acetic acid **b** with *N*,*N*-carbonyldiimidazole (CDI) (method A), or by alkylation of tetrazolo[1,5-*c*]quinazolin-5-thion potassium salt with proper halogen derivatives (method B) (Scheme 1, Table 1).



Scheme 1 Synthesis of *N*-aryl(benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides.

Table 1 Radicals of the synthesized compounds.

Compd.	*R*	R_1	*n*	Compd.	*R*	R_1	*n*
1.1	2-OCH_3	–	0	**1.18**	2-F	–	1
1.2	3-OCH_3	–	0	**1.19**	3-F	–	1
1.3	4-OCH_3	–	0	**1.20**	4-F	–	1
1.4	2-F	–	0	**1.21**	2-Cl	–	1
1.5	3-F	–	0	**1.22**	3-Cl	–	1
1.6	4-F	–	0	**1.23**	4-Cl	–	1
1.7	2-Cl	–	0	**1.24**	2-Br	–	1
1.8	3-Cl	–	0	**1.25**	3-Br	–	1
1.9	4-Cl	–	0	**1.26**	4-Br	–	1
1.10	2-Br	–	0	**1.27**	2-CF_3	–	1
1.11	3-Br	–	0	**1.28**	4-CF_3	–	1
1.12	4-Br	–	0	**1.29**	–	–	–
1.13	3-CF_3	–	0	**1.30**	–	–	–
1.14	4-CF_3	–	0	**1.31**	–	6-methyl-1,3-benzothiazol-2-yl	–
1.15	2-OCH_3	–	1	**1.32**	–	6-methoxybenzo[*d*]thiazol-2-yl	–
1.16	3-OCH_3	–	1	**1.33**	–	6-chlorobenzo[*d*]thiazol-2-yl	–
1.17	4-OCH_3	–	1				

The experiment showed that imidazolides were obtained quite easily with good yields. Acetamides **1.1**–**1.5** were also obtained by the alternative approach – alkylation of potassium salt **a** with *N*-aryl-2-chloroacetamides (method B) in water–propanol-2 mixture (2:1). The last method has a range of advantages, notably, short duration of time (60–90 min), quantitative yields and highly pure products. The structure of all synthesized compounds was evaluated by elemental analysis and their spectral data (FT-IR, ^{1}H, LC–MS spectra).

LC–MS data confirmed the purity of obtained substances, demonstrating their appropriately protonated molecular ions $[M+H]^+$.

FT-IR spectra of *N*-aryl(benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides (**1.1**–**1.33**) were characterized by high intensive stretching vibrations of symmetric and asymmetric deformational vibrations of the CH_2 group that appeared at 2952–2847 and 1469–1491 cm^{-1} appropriately. Amides had intense vibrations of the C=O group, which were registered in the range of 1636 to 1684 cm^{-1}, and partially overlapped stretchings of the NH-group, that were observed at 1619–1636 cm^{-1}, and appeared as a doublet. The stretching vibrations assigned to the NH-band occured in the range of 3353 to 3258 cm^{-1}. In addition, amides gave relatively strong bending band at 1555–1556 cm^{-1}, due to the combination of CN- and NH-vibrations. Moderate absorption at 1319–1106 cm^{-1} was caused by stretching vibrations of C-C-C, C-C(=O)-C and C-C-C. All other registered peaks characterized the condensed aromatic rings: ν_{CH} (3108–3016 cm^{-1}), ν_{CH} (902–649 cm^{-1}), ν_{CS} (713–604 cm^{-1}), ν_{CS} and ν_{CN} (1593–710 cm^{-1}).

The signals in the ^{1}H NMR spectra of the synthesized compounds appeared in accordance with the proposed structure: one-proton doublet of H-10 at 8.57–8.51 ppm, two-proton multiplet of H-7 and H-9 at 8.03–7.94 ppm and one-proton triplet of H-8 at 7.83–7.78 ppm, like in the previous results (Antypenko et al., 2013; Hand et al., 1984). However, for many compounds aromatic protons of quinazoline were overlapped with protons of phenyl- or benzyl-radicals. For compounds with phenyl moiety (**1.1**–**1.14**) NH-proton was observed as a singlet at 10.72–9.56 ppm, and for substances with benzyl fragment (**1.15**–**1.21**, **1.23**–**1.28**) – as an unsplit triplet at 8.82–8.75 ppm. For heteryl substituted substances NH-proton was shifted to a weaker field and was detected at

Table 2 Values of BL in acute action test (%). The substances with bold values have shown the most significant activities.

Compd.[a]	Concentration (mg/mL)			
	0	**0.025**	**0.1**	**0.25**
1.1	100.0	62.3	39.0	42.9
1.2	100.0	87.0	52.2	17.4
1.3	100.0	**49.1**	**5.8**	**0.0**
1.4	100.0	**39.58**	**4.17**	**0.0**
1.5	100.0	52.2	8.7	0.0
1.6	100.0	**32.2**	**6.2**	**2.5**
1.7	100.0	58.0	32.4	0.0
1.8	100.0	85.7	47.9	15.1
1.9	100.0	60.0	5.3	1.8
1.10	100.0	**25.7**	**7.4**	**3.7**
1.11	100.0	90.8	32.8	15.1
1.12	100.0	76.3	27.7	13.9
1.13	100.0	99.2	34.6	11.5
1.14	100.0	79.3	45.9	27.3
1.15	100.0	**124.0**	**21.1**	**3.7**
1.16	100.0	78.9	58.4	44.2
1.17	100.0	74.0	18.2	77.9
1.18	100.0	73.8	92.3	55.4
1.19	100.0	82.9	95.3	7.1
1.20	100.0	80.6	80.6	80.6
1.21	100.0	**139.1**	**130.4**	**87.0**
1.22	100.0	98.0	91.5	22.9
1.23	100.0	**111.5**	**86.0**	**89.2**
1.24	100.0	98.0	35.9	22.9
1.25	100.0	**107.9**	**80.6**	**83.1**
1.26	100.0	93.75	52.08	20.83
1.27	100.0	90.9	75.3	45.5
1.28	100.0	3.7	22.1	11.0
1.29	100.0	3.2	54.1	54.1
1.30	100.0	66.2	67.5	62.3
1.32	100.0	64.6	41.5	23.1
1.33	100.0	63.5	56.5	8.8
Tetracycline	100.0	80.7	9.1	0.0
DMSO (control)	100.0	141.7	119.6	110.4

[a] Substance **1.31** was not tested.

Table 3 Values of BL in chronic action test (%). The substances with bold values have shown the most significant activities.

Compd.[a]	Concentration (mg/mL)			
	0	**0.025**	**0.1**	**0.25**
1.1	100.0	23.1	53.8	92.3
1.2	100.0	159.2	36.7	26.9
1.3	100.0	40.0	52.0	18.0
1.4	100.0	**362.8**	**395.3**	**0.0**
1.5	100.0	122.4	139.6	134.7
1.6	100.0	12.0	80.0	180.0
1.7	100.0	**185.7**	**392.9**	**642.9**
1.8	100.0	27.9	62.8	139.5
1.9	100.0	114.8	32.8	0.0
1.10	100.0	**287.4**	**86.2**	**0.0**
1.11	100.0	45.3	94.2	191.9
1.12	100.0	22.6	11.3	5.7
1.13	100.0	39.5	75.0	136.4
1.14	100.0	36.0	36.0	28.0
1.15	100.0	32.0	20.0	48.0
1.16	100.0	151.7	236.0	188.8
1.17	100.0	**323.1**	**769.2**	**669.2**
1.18	100.0	21.8	36.8	20.5
1.19	100.0	**232.8**	**98.4**	**85.2**
1.20	100.0	80.0	76.0	240.0
1.21	100.0	0.0	46.5	24.5
1.22	100.0	**187.5**	**625.0**	**750.0**
1.23	100.0	89.2	122.2	75.8
1.24	100.0	112.5	187.5	62.5
1.25	100.0	28.0	84.0	332.0
1.26	100.0	51.2	465.1	465.1
1.27	100.0	**61.5**	**769.2**	**384.6**
1.28	100.0	0.0	287.4	287.4
1.29	100.0	79.5	122.2	14.7
1.30	100.0	**84.6**	**46.2**	**107.7**
1.32	100.0	40.9	5.5	5.5
1.33	100.0	111.5	29.5	23.0
Tetracycline	100.0	0	0	0
DMSO (control)	100.0	74.5	127.7	127.7

[a] Substance **1.31** was not tested.

13.06–12.57 ppm. Interestingly, that for compound **1.22** NH proton was registered as a triplet at 8.81 ppm. For compounds **1.16**, **1.18**–**1.20**, **1.21**–**1.27** proton signals of the NHCH_2-group were slightly deshielded by electronegativity of nitrogen and appeared as a two-proton doublet at 4.43–4.32 ppm. Protons of SCH_2 were deshielded by the carbonyl group and, according to the published data, should appear at 2.10–2.50 ppm, but through the influence of sulfur signals were shifted to a weaker field. So, for compounds **1.1**–**1.14** protons of the SCH_2 group were recorded as a two-proton singlet at 4.34–4.25 ppm, and for compounds **1.15**–**1.28** at 4.55–4.43 ppm. For compounds with heteryl moiety signal of the SCH_2 group was also shifted to a weaker field to 4.81–4.55 ppm. Signals of methoxy and methyl substituents of synthesized compounds were observed in a strong field at 3.82–3.71 ppm and 2.45 ppm respectively.

4.2. Pharmacology

4.2.1. P. leiognathi bioluminescence inhibition

Among the existing bioassays marine luminous bacteria *P. leiognathi* Sh1 rank special place, which combine the advantages of bioassay and instrumental methods of the analytical signal recording. In this bioassay toxicity is determined by the change in the intensity of bioluminescence, which is a quantitative indicator of bacterial cell vital functions and makes it possible to evaluate the integral effect of the synthesized *N*-aryl(benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides on the living organisms. So, inhibition of bioluminescence (BL) in acute (Table 2) and chronic action tests (inhibiting BL and growth, Table 2) was studied.

The results of bioluminescence research showed that luminescent bacteria were sensitive to synthesized compounds in both tests (Tables 2 and 3). Among *N*-phenyl-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides compounds **1.3**, **1.4**, **1.6**, **1.10**, **1.15** had the highest inhibition properties (inhibition of the growth up to 50%) at concentration 0.025 mg/ml in acute test. It is interesting, that only *N*-benzyl substituted compounds, namely **1.15**, **1.21**, **1.23**, and **1.25** at concentration 0.025 mg/ml possessed the promotion of BL while introduction

Table 4 Antimicrobial activity of compounds (100 μg). The substances with bold values have shown the most significant activities.

Compd.	Conc., μg	Microorganism/inhibition zone diameter (mm)						
		EC[a]	SA	EA	EF	PA	KP	CA
1.1	100	6*	6	6	6	6	**8**	6
1.5	100	6	6	6	6	6	**8**	6
1.10	100	6	6	6	6	6	**7**	6
1.31	100	6	6	6	6	6	**7**	6
1.33	100	6	6	6	6	6	**8**	6
Ampicillin	10	26	22	16	17	–*	–	–
Ceftazide	30	29	21	–	–	25	–	–
Amikacin	30	21	22	–	–	27	–	–
Gentamycin	10	19	22	7	9	28	–	–
Ceftriaxone	30	33	33	–	–	25	25	–
Nystatin	100	-	–	–	–	–	–	21

[a] *Escherichia coli* (EC), *Staphylococcus aureus* (SA), *Enterobacter aerogenes* (EA), *Enterococcus faecalis* (EF), *Pseudomonas aeruginosa* (PA), *Klebsiella pneumoniae (KP)*, *Candida albicans (CA)*.

* «6» – disk diameter, mm; «–» – not done. Other synthesized substances exhibited no antimicrobial and antifungal activities in the given concentration.

Table 5 Percentage of *in vitro* tumor cell line growth at 10 μM for compounds.

Compd.	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %*
1.1	100.14	78.28–128.35	88.59 (CCRF-CEM/L), 92.21 (HOP-62/nscLC), 91.76 (NCI-H226/nscLC), 93.28 (NCI-H322 M/nscLC), 93.34 (HCT-15/CC), 91.19 (SF-295/CNSC), 95.12 (SNB-75/CNSC), 93.01 (UACC-62/M), 94.47 (OVCAR-8/OC), 78.28 (UO-31/RC), 93.06 (PC-3/PC), 94.67 (MDA-MB-231/ATCC/BC), 95.09 (T-47D/BC)
1.2	101.75	85.58–127.13	88.47 (CCRF-CEM/L), 94.61 (NCI-H322 M/nscLC), 95.93 (HCT-116/CC), 93.84 (SNB-19/CNSC), 87.63 (SNB-75/CNSC), 91.76 (UACC-62/M), 95.27 (OVCAR-8/OC), (A498/ATCC/RC) 90.23, 85.58 (UO-31/RC), 93.39 (MCF7/BC)
1.3	102.53	83.30–115.54	94.98 (CCRF-CEM/L), 93.79 (HL-60(TB)/L), 93.44 (NCI-H226/nscLC), 90.49 (NCI-H322 M/nscLC), 93.66 (SNB-19/CNSC), 88.22 (SNB-75/CNSC), 93.78 (U251/CNSC), 95.66 (UACC-257/M), 91.32 (A498/ATCC/RC), 95.34 (RXF 393/RC), 93.63 (SN12C/RC), 91.62 (UO-31/RC), 94.75 (MCF7/BC), 83.30 (BT-549/BC), 87.52 (T-47D/BC)
1.5	101.12	82.16–124.56	93.39 (CCRF-CEM/L), 92.90 (HL-60(TB)/L), 93.84 (SR/L), 84.20 (HOP-62/nscLC), 95.27 (HCT-15/CC), 93.41 (SF-295/CNSC), 95.83 (U251/CNSC), 92.63 (UACC-257/M), 94.40 (UACC-62/M), 90.56 (OVCAR-8/OC), 93.28 (CAKI-1/RC), 82.16 (UO-31/RC), 94.50 (PC-3/PC), 92.89 (MCF7/BC), 95.15 (MDA-MB-231/ATCC/BC)
1.6	100.50	60.11–129.44	86.19 (NCI-H226/nscLC), 84.70 (HCT-116/CC), 89.15 (HCT-15/CC), 94.07 (SF-539/CNSC), 95.97 (U251/CNSC), 60.11 (LOX IMVI/M), 91.44 (A498/ATCC/RC), 91.74 (ACHN/RC), 94.84 (CAKI-1/RC), 95.46 (SN12C/RC), 71.88 (MCF7/BC), 95.28 (MDA-MB-231/ATCC/BC), 94.31 (BT-549/BC), 80.53 (MDA-MB-468/BC)
1.7	101.41	80.85–120.07	89.96 (CCRF-CEM/L), 95.83 (RPMI-8226/L), 92.55 (A549/nscLC), 89.53 (HCT-15/CC), 95.21 (SF-295/CNSC), 94.61 (U251/CNSC), 95.60 (LOX IMVI/M), 93.61 (SK-MEL-5/M), 95.81 (OVCAR-8/OC), 89.97 (RXF 393/RC), 92.03 (UO-31/RC), 95.00 (BT-549/BC), 84.95 (T-47D/BC), 80.85 (MDA-MB-468/BC)
1.8	98.10	76.42–115.07	91.60 (CCRF-CEM/L), 79.26 (HOP-62/nscLC), 85.92 (NCI-H226/nscLC), 89.54 (NCI-H322 M/nscLC), 95.78 (HCT-15/CC), 92.35 (SF-539/CNSC), 93.24 (SNB-19/CNSC), 89.42 (SNB-75/CNSC), 93.70 (UACC-257/M), 86.60 (UACC-62/M), 89.35 (IGROV1/OC), 94.71 (OVCAR-4/OC), 95.86 (OVCAR-5/OC), 80.06 (A498/ATCC/RC), 92.33 (ACHN/RC), 77.45 (CAKI-1/RC), 84.21 (SN12C/RC), 76.42 (UO-31/RC), 95.85 (PC-3/PC), 81.74 (MCF7/BC), 79.07 (MDA-MB-231/ATCC/BC), 86.23 (MDA-MB-468/BC)
1.9	97.62	81.33–121.49	85.03 (CCRF-CEM/L), 85.83 (HL-60(TB)/L), 90.99 (K-562/L), 91.87 (SR/L), 81.33 (HOP-62/nscLC), 88.99 (NCI-H226/nscLC), 89.18 (SNB-75/CNSC), 93.15 (U251/CNSC), 88.41 (LOX IMVI/M), 95.00 (SK-MEL-5/M), 90.93 (UACC-62/M), 91.39 (IGROV1/OC), 93.23 (OVCAR-4/OC), 95.33 (OVCAR-5/OC), 90.79 (OVCAR-8/OC), 89.17 (MCF7/BC), 89.14 (MDA-MB-231/ATCC/BC), 90.13 (T-47D/BC)
1.16	99.68	78.07–113.52	78.07 (CCRF-CEM/L), 95.61 (K-562/L), 88.15 (MOLT-4/L), 92.03 (RPMI-8226/L), 82.45 (SR/L), 79.68 (HOP-62/nscLC), 93.92 (SF-295/CNSC), 89.46 (SK-MEL-2/M), 95.68 (CAKI-1/RC), 76.81 (UO-31/RC), 92.39 (MCF7/BC)
1.17	102.65	83.68–130.77	83.68 (HL-60(TB)/L), 95.94 (K-562/L), 90.63 (SR/L), 95.03 (HCT-15/CC), 90.44 (SNB-75/CNSC), 93.13 (SK-MEL-28/M), 92.72 (SN12C/RC), 86.82 (T-47D/BC)
1.18	99.07	75.07–128.82	91.73 (CCRF-CEM/L), 89.32 (HL-60(TB)/L), 82.41 (K-562/L), 84.98 (MOLT-4/L), 83.69 (SR/L), 79.85 (HOP-62/nscLC), 88.86 (HCT-116/CC), 92.90 (SNB-75/CNSC), 94.91 (LOX IMVI/M), 91.70 (SK-MEL-2/M), 87.57 (SK-OV-3/OC), 93.47 (ACHN/RC), 93.57 (CAKI-1/RC), 92.00 (TK-10/RC), 75.07 (UO-31/RC), 92.27 (PC-3/PC), 94.14 (MCF7/BC), 94.05 (T-47D/BC)
1.19	97.98	38.33–119.50	81.21 (CCRF-CEM/L), 90.07 (HL-60(TB)/L), 95.43 (K-562/L), 89.44 (RPMI-8226/L), 95.91 (SR/L), 87.47 (HCT-116/CC), 94.79 (HCT-15/CC), 91.33 (SNB-75/CNSC), 38.33 (LOX IMVI/M), 90.35 (UACC-257/M), 94.10 (UACC-62/M), 94.28 (OVCAR-4/OC), 95.67 (ACHN/RC), 92.65 (SN12C/RC), 94.35 (UO-31/RC), 86.47 (PC-3/PC), 85.30 (MCF7/BC), 95.77 (T-47D/BC), 89.42 (MDA-MB-468/BC)
1.20	96.52	78.61–115.94	92.60 (CCRF-CEM/L), 86.71 (HL-60(TB)/L), 91.33 (K-562/L), 85.20 (RPMI-8226/L), 92.38 (NCI-H226/nscLC), 91.48 (HCT-116/CC), 94.98 (HCT-15/CC), 90.11 (HT29/CC), 93.58 (SNB-75/CNSC), 90.85 (U251/CNSC), 82.96 (LOX IMVI/M), 95.29 (MALME-3 M/M), 90.69 (M14/M), 92.94 (SK-MEL-5/M), 86.96 (UACC-257/M), 93.67 (IGROV1/OC), 89.72 (OVCAR-4/OC), 91.21 (OVCAR-8/OC), 91.13 (786–0/RC), 92.85 (A498/RC), 83.14 (RXF 393/RC), 95.23 (UO-31/RC), 81.22 (PC-3/PC), 93.22 (MCF7/BC), 94.97 (BT-549/BC), 78.61 (MDA-MB-468/BC)
1.23	99.58	79.68–125.33	79.68 (HL-60(TB)/L), 88.13 (K-562/L), 95.47 (HCT-116/CC), 95.95 (HCT-15/CC), 95.82 (SF-539/CNSC), 93.54 (U251/CNSC), 91.98 (OVCAR-4/OC), 85.17 (UO-31/RC), 90.05 (PC-3/PC), 95.74 (MCF7/BC), 93.93 (MDA-MB-231/ATCC/BC), 94.99 (BT-549/BC), 86.44 (T-47D/BC), 87.50 (MDA-MB-468/BC)

(*continued on next page*)

Table 5 (*continued*)

Compd.	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %*
1.29	95.51	70.31–114.09	77.61 (CCRF-CEM/L), 95.56 (MOLT-4/L), 84.37 (RPMI-8226/L), 93.13 (SR/L), 79.63 (HOP-62/nscLC), 91.73 (NCI-H226/nscLC), 95.40 (HCT-116/CC), 93.05 (KM12/CC), 85.29 (SF-295/CNSC), 95.63 (SF-539/CNSC), 78.17 (SNB-75/CNSC), 91.85 (LOX IMVI/M), 95.90 (MALME-3 M/M), 93.22 (UACC-257/M), 95.83 (UACC-62/M), 83.08 (IGROV1/OC), 91.03 (SK-OV-3/OC), 81.51 (CAKI-1/RC), 90.46 (SN12C/RC), 70.31 (UO-31/RC), 71.81 (MCF7/BC), 95.57 (MDA-MB-231/ATCC/BC), 85.34 (T-47D/BC), 79.75 (MDA-MB-468/BC)
1.30	96.97	72.69–122.59	72.69 (CCRF-CEM/L), 86.01 (HL-60(TB)/L), 89.84 (K-562/L), 91.05 (MOLT-4/L), 92.57 (RPMI-8226/L), 83.80 (SR/L), 94.03 (A549/nscLC), 95.28 (NCI-H322 M/nscLC), 95.08 (HCT-116/CC), 91.77 (HCT-15/CC), 92.29 (SF-539/CNSC), 91.25 (MDA-MB-435/M), 95.16 (UACC-257/M), 94.03 (UACC-62/M), 88.50 (OVCAR-8/OC), 92.78 (786–0/RC), 91.86 (ACHN/RC), 91.58 (CAKI-1/RC), 86.99 (RXF 393/RC), 75.37 (UO-31/RC), 90.52 (MCF7/BC), 94.56 (BT-549/BC), 76.33 (T-47D/BC), 89.16 (MDA-MB-468/BC)
1.31	93.51	31.40–124.47	87.80, (CCRF-CEM/L), 85.60 (RPMI-8226/L), 95.58 (SR/L), 89.56 (HOP-62/nscLC), 82.98 (NCI-H226/nscLC), 87.27 (NCI-H322 M/nscLC), 92.58 (NCI-H460/nscLC), 94.26 (HCT-116/CC), 89.31 (HCT-15/CC), 89.01 (KM12/CC), 31.40 (LOX IMVI/M), 94.58 (M14/M), 87.93 (SK-MEL-2/M), 81.44 (SK-MEL-5/M), 83.78 (UACC-62/M), 89.86 (IGROV1/OC), 95.63, (OVCAR-3/OC), 87.67 (OVCAR-4/OC), 91.94 (OVCAR-5/OC), 92.20 (SK-OV-3/OC), 87.37 (ACHN/RC), 91.45 (CAKI-1/RC), 93.76 (SN12C/RC), 76.58 (UO-31/RC), 91.03 (PC-3/PC), 64.41 (MCF7/BC), 69.95 (MDA-MB-231/ATCC/BC), 73.65 (T-47D/BC), 70.98 (MDA-MB-468/BC)
1.32	83.53	−29.66–120.96	70.35 (CCRF-CEM/L), 86.96 (HL-60(TB)/L), 80.44 (K-562/L), 75.53 (MOLT-4/L), 77.38 (RPMI-8226/L), 47.33 (SR/L), 64.43 (A549/nscLC), 81.25 (HOP-62/nscLC), 92.06 (NCI-H23/nscLC), 86.46 (NCI-H322 M/nscLC), 38.23 (NCI-H460/nscLC), 95.60 (HCC-2998/CC), 79.41 (HCT-15/CC), 31.50 (KM12/CC), 87.91 (SF-268/CNSC), 16.79 (SF-295/CNSC), 88.85 (SF-539/CNSC), 92.80 (SNB-19/CNSC), 68.01 (U251/CNSC), -29.66 (LOX IMVI/M), 84.06 (M14/M), 92.05 (MDA-MB-435/M), 77.13 (SK-MEL-2/M), 84.52 (UACC-257/M), 73.63 (UACC-62/M), 95.28 (OVCAR-5/OC), 81.75 (OVCAR-8/OC), 87.52 NCI/ADR-RES, 85.99 (SK-OV-3/OC), 85.75 (ACHN/RC), 65.32 (CAKI-1/RC), 87.33 (SN12C/RC), 71.80 (UO-31/RC), 82.16 (PC-3/PC), 95.48 (DU-145/PC), 72.20 (MCF7/BC), 71.08 (MDA-MB-231/ATCC/BC), 82.45 (T-47D/BC)
1.33	91.30	56.13–113.40	75.35 (CCRF-CEM/L), 80.67 (HL-60(TB)/L), 79.88 (K-562/L), 74.47 (MOLT-4/L), 80.58 (RPMI-8226/L), 73.40 (SR/L), 83.00 (A549/nscLC), 87.28 (HOP-62/nscLC), 93.32 (NCI-H23/nscLC), 94.75 (NCI-H322 M/nscLC), 81.77 (NCI-H460/nscLC), 92.50 (HCT-15/CC), 56.13 (KM12/CC), 93.64 (SF-268/CNSC), 68.87 (SF-295/CNSC), 92.83 (SNB-19/CNSC), 89.68 (SNB-75/CNSC), 82.71 (U251/CNSC), 95.79 (LOX IMVI/M), 90.71 (MDA-MB-435/M), 84.20 (SK-MEL-2/M), 94.75 (UACC-257/M), 82.20 (UACC-62/M), 89.79 (IGROV1/OC), 90.74 (OVCAR-4/OC), 95.27 (OVCAR-5/OC), 92.61 (OVCAR-8/OC), 88.45 (SK-OV-3/OC), 89.77 (ACHN/RC), 82.09 (CAKI-1/RC), 83.77 (SN12C/RC), 73.58 (UO-31/RC), 93.30 (PC-3/PC), 69.78 (MCF7/BC), 78.78 (MDA-MB-231/ATCC/BC), 3.07 (T-47D/BC), 89.31 (MDA-MB-468/BC)

BC, breast cancer; CC, colon cancer; CNSC, CNS cancer; L, leukemia; M, melanoma; nscLC, non-small cell lung cancer; OC, ovarian cancer; PC, prostate cancer; RC, renal cancer.

of the heteryl substituents caused the moderate inhibition properties.

According to the chronic action test *N*-benzyl-substituted compounds **1.4**, **1.7**, **1.10**, **1.17**, **1.19**, **1.22** and **1.27** exhibited significant promotive BL activity. Such, for compound **1.22** percentage of BL at concentration 0.25 mg/ml was 750% up to 127.7% of control. It should be noted, that compounds **1.1**, **1.6**, **1.8**, **1.11**, **1.13**, **1.21** and **1.28** in the chronic test showed effect of hormesis, inhibiting the intensity of bioluminescence in the smallest concentration (0.025 mg/ml).

Thus, the majority of the substances had negative effect on the bacteria *P. leiognathi* Sh1 BL in acute and chronic action tests, showing toxicity, but the other part demonstrated BL high stimulation. In comparison to the previous investigated introduction of carboxylic acid or aromatic acid ester, 2-methyl-4-nitrophenyl and ethanone radicals (Antypenko et al., 2013), substituted with amides compounds had less toxic effect.

4.2.2. Antimicrobial and antifungal activities

All synthesized compounds were evaluated for their *in vitro* antibacterial activity against Gram positive bacteria (*S. aureus*, *E. faecalis*), Gram negative bacteria (*E. aerogenes*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*) and antifungal properties against *C. albicans*. The agar-diffusion method was used to determine the preliminary activity compared to well known reference antimicrobials. The compounds were dissolved in concentration of 100 μg/disk in DMSO. And inhibition zone diameter

(IZD, mm) was used as a measure for the antimicrobial activity (Table 4).

Synthesized compounds have revealed neither high antibacterial, nor antifungal activities. It was found that compounds **1.1**, **1.5**, **1.10**, **1.31**, **1.33** possessed light activity against *Klebsiella pneumonia*.

Thus, introduction of acetamide moiety into the 5th position of tetrazolo[1,5-*c*]quinazoline molecule had negative impact on its antimicrobial properties, comparing the previous demonstration of the antibacterial activity against *E. faecalis*, *S. aureus* and *E. coli*, and antifungal activity against *C. albicans*, when ethylaminodialkyl and chloropropyl substituents were presented in its structure (Antypenko et al., 2013).

4.2.3. Anticancer assay for preliminary in vitro testing

Among all of the newly synthesized compounds 19 substances, namely **1.1**–**1.3**, **1.5**–**1.9**, **1.16**–**1.20**, **1.23**, **1.29**–**1.33**, were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program for the *in vitro* cell line screening to investigate their anticancer activity (Boyd and Paull, 1995; Boyd, 1997). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. Initially, a single high concentration was used (10 μM) in the full NCI 60-cell panel. In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Then test substances were added to the plate and the culture was incubated for further 48 h. End point determinations were made with a protein binding dye, sulforhodamine B. Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells (Table 5).

The antitumor activity of the compounds was measured according to a value of 100 that meant no growth inhibition. A value of 30 would mean 70% growth inhibition. A value of 0 meant no net growth over the course of the experiment. A value of −30 would mean 70% lethality. A value of −100 meant all cells were dead.

N -Heteryl-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides were found to have the strongest anticancer activity. Thus, *N*-(benzo[*d*]thiazol-2-yl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio) acetamides revealed the highest inhibition properties against cell of melanoma. Introducing the methoxy group in the C-6 position of benzo[*d*]thiazole led to the most active compound *N*-(6-methoxybenzo[*d*]thiazol-2-yl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamide (**1.32**), that showed lethal antitumor activity (10 μM) against cell line LOX IMVI of melanoma. Replacement of the methoxy group by the methyl group (**1.31**) led to the decrease of anticancer activity, and introduction of chlorine to the benzo[*d*]thiazole (**1.33**) caused the loss of the property. Compound **1.32** besides negative melanoma influence demonstrated considerable anticancer activity against leukemia cell line SR to 47.33%, nscLC cell line NCI-H460 to 38.23%, colon cancer KM12 cell line to 31.50% and CNS cancer SF-295 cell line to 16.79%.

Speaking about phenyl- and benzyl-substituted compounds, *N*-(3-fluorobenzyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamide (**1.19**) exhibited high inhibition growth percent (38.33%) against cell line LOX IMVI of melanoma. Phenyl-substituted compounds exhibited only moderate anticancer effect.

So, it was proved, that *N*-aryl(benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides were selective against cell line LOX IMVI of melanoma, unlike the leukemia CCRF-CEM cell line of 2-(tetrazolo[1,5-c]quinazolin-5-ylthio)-1-(p-tolyl)ethanone and corresponding substituted carboxylic acids of previous research (Antypenko et al., 2013). But, unfortunately, all of these compounds still have not satisfied the predetermined threshold inhibition criteria to progress to the 5-dose screen, which was designed to efficiently capture compounds with antiproliferative activity.

4.2.4. Anticancer docking

Knowing that novel 4-anilinoquinazolines (Barbosa et al., 2014; Shuang et al., 2013), that contained quinazoline moiety, inhibited EGFR and were analyzed for molecular docking, ATP-binding site of EGFR (2ITY.pdb) was selected for the docking study to show potential anticancer mechanism of *N*-aryl(benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio) acetamides. Research was conducted by the flexible molecular docking using the software package OpenEye, including related utilities: Fred Receptor2.2.5, Vida4.1.1, Flipper, Babel3, Omega2.4.3 and Fred2.2.5 (OpenEye Scientific Software, 2005). The crystal structure of the enzyme with known quinazoline derivative anticancer drug Gefitinib was obtained from the protein data bank.

Docking methodology is described in our recent investigation (Kovalenko et al., 2013; Antypenko et al., 2013). Scoring functions, which were used for calculating the affinity, are present in Table 6.

According to Consensus score compounds *N*-(2-methoxyphenyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamide (**1.1**) and *N*-(2-fluorobenzyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio) acetamide (**1.18**) revealed the highest affinity to a specific biological target, but still lower compared with Gefitinib and Lapatinib. Moreover, molecular docking with visual inspection indicated, that compound **1.18** was bounded to the EGFR kinase with hydrogen bond: C(O···MET:793:A (2.36 Å) (Fig. 1). Yet, preliminary anticancer studies showed, that this compound did not reveal significant activity.

Compound **1.32**, the most active one according to the *in vitro* cell line screening in the National Cancer Institute

Table 6 Scoring functions of docked compounds with best affinity compared to Gefitinib and Lapatinib.

Compd.	Consensus score	Shape gauss	PLP	Chem gauss2	Chem gauss3	Chem score	OEchem score	Screen score	CGO	CGT	Zapbind
Gefitinib	9.00	−507.26	−56.55	−59.78	−69.13	−20.41	−35.31	−126.06	−367.45	−0.61	−25.60
Lapatinib	59.00	−599.98	−61.50	−64.46	−71.10	−16.22	−42.82	−131.70	−329.62	−0.43	−29.62
1.18	89.00	−451.92	−47.48	−49.47	−59.70	−13.36	−29.37	−101.90	−317.69	−0.54	−24.61
1.1	103.00	−453.86	−47.75	−46.66	−56.90	−19.36	−33.68	−107.47	−293.39	−0.53	−20.66
1.32	194.00	−451.24	−38.31	−43.75	−53.97	−14.71	−25.50	−100.40	−291.95	−0.42	−24.71
1.31	194.00	−418.91	−43.01	−40.97	−48.96	−14.98	−27.02	−99.32	−287.15	−0.52	−24.69

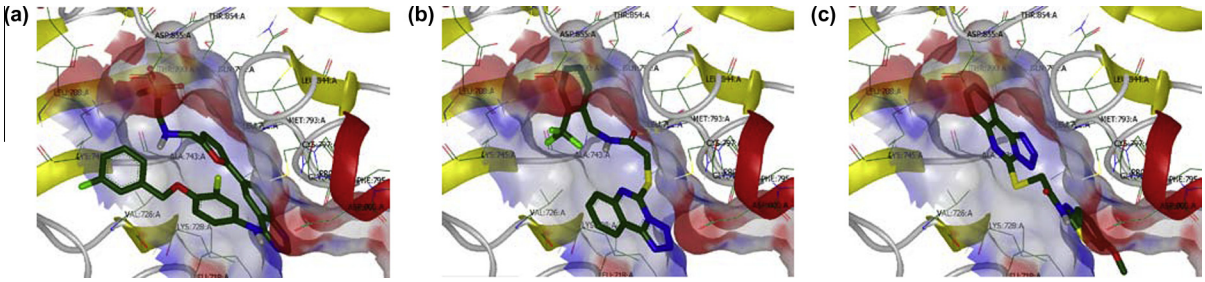


Figure 1 (a) Interaction of Lapatinib with the binding site of EGFR; (b–c) Interaction of compound **1.18**, **1.32** with the binding site of EGFR. H-bond is shown as a yellow line.

(NCI), had the moderate data of Consensus score. Considering these results, it can be assumed, that the most active anticancer substances' mechanism of action is of a different nature. Though, there is a prediction of high affinity of (tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides to the EGFR kinase for their future anticancer investigations.

5. Conclusions

The efficient procedures for the synthesis of novel *N*-aryl (benzyl,heteryl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides were proposed. The substances structures were elucidated by FT-IR, LC–MS, ^{1}H NMR and elemental analysis data. The results of bioluminescence research showed that luminescent bacteria *P. leiognathi* strain Sh1 were sensitive to synthesized compounds in acute and chronic action tests, showing bacteriostatic effect. Compounds **1.6**–**1.8**, **1.11**, **1.13** and **1.22** in the chronic test showed effect of hormesis, inhibiting the intensity of bioluminescence in the smallest concentration (0.025 mg/ml). The subsequent screening for antibacterial and antifungal activities in concentration 100 μg revealed light selective effect against *Klebsiella pneumonia* of substances **1.1**, **1.5**, **1.10**, **1.31**, **1.33**. Anticancer assay for preliminary (10 μM) *in vitro* testing in NCI showed, that *N*-(benzo[*d*]thiazol-2-yl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides possessed the highest inhibition properties against cells of melanoma. Compound **1.32** possessed lethal antitumor activity against LOX IMVI cell line of melanoma and **1.31** inhibited its growth to 31.40%. Benzyl *N*-3-fluorobenzylsubstituted **1.19** exhibited high inhibition growth percent (38.33%) against the same cell line. So, *N*-benzyl-(heteryl-)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides appeared to be the promising anticancer agents against melanoma. Molecular docking studies into the ATP-binding site of EGFR indicated that compounds could act like Gefitinib, though the strongest anticancer substances had not the highest Consensus score data. Hence, purposeful design of anticancer tetrazolo[1,5-*c*]quinazoline derivatives is going to be continued among other *N*-heterylsubstituted acetylamides.

Acknowledgements

The authors gratefully acknowledge "Enamine Ltd." (Kiev, Ukraine) for financial support of this work, and team of the Drug Synthesis and Chemistry Branch, National Cancer Institute, (Bethesda, MD, USA) for *in vitro* evaluation of anticancer activity.

References

Antipenko, L.N., Karpenko, A.V., Kovalenko, S.I., Katsev, A.M., Komarovska-Porokhnyavets, E.Z., Novikov, V.P., 2009a. Synthesis, cytotoxicity by bioluminescence inhibition, antibacterial and antifungal activity of ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acid amides. Arch. Pharm. Chem. Life Sci. 342, 651–662.

Antipenko, L.N., Karpenko, A.V., Kovalenko, S.I., Katsev, A.M., Komarovska-Porokhnyavets, E.Z., Novikov, V.P., Chekotilo, A., 2009b. Synthesis of new 2-thio-[1,2,4]triazolo[1,5-*c*]quinazoline derivatives and its antimicrobial activity. Chem. Pharm. Bull. 57 (6), 580–585.

Antypenko, L.M., Kovalenko, S.I., Antypenko, O.M., Katsev, A.M., Achkasova, O.M., 2013. Design and evaluation of novel antimicrobial and anticancer agents among tetrazolo[1,5-*c*]quinazoline-5-thione S-derivatives. Sci. Pharm. 81, 15–42.

Barbosa, M.L., Lima, L.M., Tesch, R., Sant'Anna, C.M., Totzke, F., Kubbutat, M.H., Schächtele, C., Laufer, S.A., Barreiro, E.J., 2014. Novel 2-chloro-4-anilino-quinazoline derivatives as EGFR and VEGFR-2 dual inhibitors. Eur. J. Med. Chem. 71, 1–14.

Berest, G.G., Voskoboynik, O.Y., Kovalenko, S.I., Antypenko, O.M., Nosulenko, I.S., Katsev, A.M., Shandrovskaya, O.S., 2011. Synthesis and biological activity of novel *N*-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetamides. Eur. J. Med. Chem. 46, 6066–6074.

Berest, G.G., Voskoboynik, O.Y., Kovalenko, S.I., Nosulenko, I.S., Antypenko, L.M., Antypenko, O.M., Shvets, V.M., Katsev, A.M., 2012. Synthesis of new 6-{[ω-(dialkylamino(heterocyclyl)alkyl]thio}-3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones and evaluation of their anticancer and antimicrobial activities. Sci. Pharm. 80, 37–65.

Boyd, M.R., Paull, K.D., 1995. Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen. Drug Dev. Res. 34, 91–109.

Boyd, M.R., 1997. The NCI In Vitro Anticancer Drug Discovery Screen. Concept, Implementation and Operation. Humana Press 2, pp. 23–43.

Cao, C., Albert, J.M., Geng, L., Ivy, P.S., Sandler, A., Johnson, D.H., Lu, B., 2006. Vascular endothelial growth factor tyrosine kinase inhibitor azd2171 and fractionated radiotherapy in mouse models of lung cancer. Cancer Res. 66 (23), 11409–11415.

Carlomagno, F., Vitagliano, D., Guida, T., Ciardiello, F., Tortora, G., Vecchio, G., Ryan, A.J., Fontanini, G., Fusco, A., Santoro, M., 2002. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. Cancer Res. 62 (24), 7284–7290.

Ellis, G.P., West, G.B., (Eds.), 1980. Medicinal chemistry of tetrazoles. Prog. Med. Chem., Elsevier/North-Holland Biomedical Press, pp. 152–172.

Hand, E.S., Baker, D.C., 1984. A definitive synthesis of 2-[(5-methyl-s-triazolo[4,3-*c*]quinazolin-3-yl)thio]-*N*-benzylacetanilide and other reactions of its precursors. Can. J. Chem. 62, 2570–2577.

Jantova, S., Stankovský, Š., Špirková, K., 2004. In vitro antibacterial activity of ten series of substituted quinazolines. Biologia 59, 741–752.

Kanagarajan, V., Thanusu, J., Gopalakrishnan, M., 2010. Synthesis and in vitro microbiological evaluation of an array of biolabile 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides. Eur. J. Med. Chem. 45, 1583–1589.

Kategaonkar, A.H., Labade, V.B., Shinde, P.V., Kategaonkar, A.H., Shingate, B.B., 2010. Cheminform abstract: synthesis and antimicrobial activity of tetrazolo[1,5-*a*]quinoline-4-carbonitrile derivatives. Monatsh. Chem. 141, 787–791.

Konecny, G.E., Pegram, M.D., Venkatesan, N., Finn, R., Yang, G., Rahmeh, M., Untch, M., Rusnak, D.W., Spehar, G., Mullin, R.J., Keith, B.R., Gilmer, T.M., Berger, M., Podratz, K.C., Slamon, D.J., 2006. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-Overexpressing and trastuzumab-treated breast cancer cells. Cancer Res. 66 (3), 1630–1639.

Kovalenko, S.I., Nosulenko, I.S., Voskoboynik, A.Y., Berest, G.G., Antypenko, L.N., Antypenko, A.N., Katsev, A.M., 2012. Substituted 2-[(2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamides with thiazole and thiadiazole fragments: synthesis, physicochemical properties, cytotoxicity, and anticancer activity. Sci. Pharm. 80 (4), 837–865.

Kovalenko, S.I., Nosulenko, I.S., Voskoboynik, A.Y., Berest, G.G., Antypenko, L.N., Antypenko, A.N., Katsev, A.M., 2013. Novel *N*-aryl(alkaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl) thio]acetamides: synthesis, cytotoxicity, anticancer activity, COMPARE analysis and docking. Med. Chem. Res. 22, 2610–2632.

Mohammed, A.E.S., Mamdouh, A.M.T., Essam, M.S., 1991. Synthesis and biological activities of condensed heterocyclo [*n,m-a,b*, or *c*]quinazolines. Adv. Heterocycl. Chem. 52, 69–70.

Myznikov, L.V., Hrabalek, A., Koldobskii, G.I., 2007. Drugs in the tetrazole series. (Review). Chem. Heterocycl. Comp. 43 (1), 1–9.

Nosulenko, I.S., Voskoboynik, O.Yu., Berest, G.G., Safronyuk, S.L., Kovalenko, S.I., Katsev, An.V., Sinyak, R.S., 2014. Synthesis and antiviral activity of [(9-R1-10-R2-3-R-2-oxo-2*H*-[1,2,4]-triazino[2,3-*C*]quinazolin 6-yl)thio]acetamides derivatives with the fragments of carcass amines. J. Org. Pharm. Chem. 1 (45), 17–27.

Orek, C., Koparir, P., Koparir, M., 2012. *N*-cyclohexyl-2-[5-(4-pyridyl)-4-(p-tolyl)-4*H*-1,2,4-triazol-3-ylsulfanyl]-acetamide dihydrate: synthesis, experimental, theoretical characterization and biological activities. Spectrochim. Acta A 97, 923–934.

Patel, R.P., Patel, P.K., Kumari, P., Rajani, D.P., Chikhalia, K.C., 2012. Synthesis of benzimidazolyl-1,3,4-oxadiazol-2ylthio-*N*-phenyl (benzothiazolyl) acetamides as antibacterial, antifungal and antituberculosis agents. Eur. J. Med. Chem. 53, 41–51.

Rusnak, D.W., Lackey, K., Affleck, K., Wood, E.R., Alligood, K.J., Rhodes, N., Keith, B.R., Murray, D.M., Knight, W.B., Mullin, R.J., Gilmer, T.M., 2001. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. Mol. Cancer Ther. 1 (2), 85–94.

Saha, S., Priyadharshini, A., Dhanasekaran, D., Thajuddin, N., Chandraleka, S., Chandramohan, G., Panneerselvam, 2012. Preclinical evaluation and molecular docking of 4-phenyl-1-Napthyl phenyl acetamide (4P1NPA) from Streptomyces sp. DPTB16 as a potent antifungal compound A. Comput. Biol. Med. 42 (5), 542–547.

Sherif, A.F.R., Hayam, M.A.A., Heba, A.A.R., Abd, E.F.H., Abd, E.F., 2009. Azole antimicrobial pharmacophore-based tetrazoles: Synthesis and biological evaluation as potential antimicrobial and anticonvulsant agents. Bioorg. Med. Chem. 17, 2410–2422.

Shuang, L., Wei, Z., Liyun, J., Qun, L., Xiang, H., Xianchan, L., Fuyi, W., 2013. Synthesis, characterization, screening and docking analysis of 4-anilinoquinazoline derivatives as tyrosine kinase inhibitors. Eur. J. Med. Chem. 61, 84–94.

Technical documentation for OpenEye Software. Omega, Fred, OpenEye Scientific Software, 2005.