Synthesis of 6-N-R-Tetrazolo[1,5-c]quinazolin-5(6H)-ones and Their Anticancer Activity

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Abstract

Chemical compounds with tetrazole ring are very interesting systems that can be valuable in pharmaceutical and clinical applications, especially as anticancer agents. In this work, novel 6-N-R-tetrazolo[1,5-c]quinazolin-5(6H)-ones were synthesized. A large set of IR, LC-, EI-MS, 1H, 13C NMR and elemental analysis data were collected and evaluated for their structures and purity. Details of synthesis, namely the N-alkylation, are discussed, including reactions with secondary and tertiary amides. Four new synthesized compounds (2.7, 3.2, 5.2, 5.3) were tested in vitro for anticancer activity at 10 μM against 60 cell lines of nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. Further synthesis of substances within the series of substituted tetrazolo[1,5-c]quinazoline systems will be attempted to develop improved compounds with better anticancer activity.

Keywords: Anticancer activity; 6-N-R-tetrazolo[1,5-c]quinazolin-5(6H)-ones; organic synthesis

1. Introduction

Tetrazole ring is a very interesting system and chemical compounds with this ring find diverse biological, pharmaceutical, and clinical applications, despite its absence in nature. Many highly effective agents have active pharmaceutical ingredients containing the tetrazole ring. During relatively short period of time many such compounds have appeared in the world of pharmaceutical market. Thus, among the drugs with tetrazole ring and agents under trials are the following compounds: hypotensive (Losartan), antimicrobial (Cefamandol), antifungal (TAK-456), anti-inflammatory (Figure 1, a), antiviral (5-CIT-EP), antihistaminic (Tazanoplast, Planlukast), cytostatic (Figure 1, b), central nervous system influence (Corazolum), and others (Figure 1.). Also, the anticancer activity of tetrazol was recently reported. cis-[PtCl2(DMSO)L], where ligands are a Schiff base or hydrazone are derived from tetrazolo[1,5-c]quinolin-4-carboxaldehyde (Figure 1.). Moreover, fibrinolytic and bronchodilating activities of such compounds were claimed by several US patents. Our latest investigations of substituted condensed tetrazolo[1,5-c]quinazolines have also proved their potential as pharmaceutical agents, namely anticancer (N-(benzo[d]thiazol-2-yl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)acetamides against cells of melanoma), antimicrobial (1-(2,5-dimethoxyphenyl)-2-(tetrazolo[1,5-c]quinazolin-5-ylthio)ethanone against Staphylococcus aureus), antifungal (5-(3-chloropropylthio)tetrazolo[1,5-c]quinazoline against Candida albicans), and bioluminescence inhibition properties.

So, tetrazolo[1,5-c]quinazolines are of undoubting interest and valuable objects for further research. In this work, as a logical continuation of our previous investigations a range of 6-N-R-tetrazolo[1,5-c]quinazolin-5(6H)-

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IR spectrometer using a module eco ZnSe. ¹H NMR spectra (400 MHz) and ¹³C NMR spectra (100 MHz) were recorded at a Varian-Mercury 400 and Bruker Avance DRX-500 spectrometers with SiMe₃ as internal standard in DMSO-d₆ 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). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV.

2.2. Pharmacology

2.2.1. Anticancer Assay for Preliminary in vitro Testing

From the newly synthesized compounds 4 substances, namely 2.7, 3.2, 5.2, 5.3 were selected by the NCI Developmental Therapeutic Program for in vitro cell line screening to investigate their anticancer activity. 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 1).

General Procedure for the Synthesis of 6-N-R-Tetrazo[1,5-c]quinazolin-5(6H)-ones.

To a solution of 0.9 g (4.8 mmol) of tetrazo[1,5-c]quinazolin-5(6H)-one (1.1) in DMF 0.17 g (4.8 mmol) of sodium hydride (60% oil suspension) was added. After 5–10 min, when all hydrogen has been released, the appropriate halogen derivative was added (4.8 mmol). The mixture was refluxed for 2 h and cooled down. Then DMF was evaporated under vacuum and water was added to form the precipitate. It was filtered, washed with water, dried and crystallized from a mixture of propane-2-ol : water (1:1).

Tetrazo[1,5-c]quinazolin-5(6H)-one (1.1)

Yield 95.6%; mp 295–297°C; IR (cm⁻¹): 3147, 3114, 3085, 3047, 2974, 2916, 2849, 2755, 2709, 1746, 1714, 1660, 1625, 1587, 1547, 1515, 1483, 1463, 1442, 1430, 1415, 1388, 1342, 1304, 1256, 1202, 1167, 1157, 1114, 1088, 1025, 955, 969, 896, 880, 810, 783, 757, 742, 731, 709, 698, 675, 658, 623. ¹H NMR: δ (ppm) 12.68 (s, 1H, NH), 8.27 (d, J = 7.8 Hz, 1H, H-10), 7.71 (t, J = 7.7 Hz, 1H, H-9), 7.50 (d, J = 8.2 Hz, 1H, H-7), 7.42 (t, J = 7.5 Hz, 1H).

Figure 1. Structures of known tetrazole ring containing drugs available on the market and related agents in clinical trials.
Hz, 1H, H-8). LC-MS: m/z 187 [M+H]+. Anal. Calcd. for C₈H₅N₅O: C, 51.34; H, 2.69; N, 37.42. Found: C, 51.38; H, 2.63; N, 37.46.

6-Methyltetrazolo[1,5-c]quinazolin-5(6H)-one (2.1)
Yield 57.0%; mp 224–226 °C; IR (cm⁻¹): 1726, 1650, 1620, 1588, 1555, 1518, 1489, 1454, 1423, 1398, 1340, 1315, 1299, 1259, 1235, 1173, 1130, 1087, 1042, 1013, 1001, 965, 877, 787, 761, 710, 674, 633. ¹H NMR: δ (ppm) 8.39 (d, J = 7.6 Hz, 1H, H-10), 7.87 (t, J = 7.5 Hz, 1H, H-9), 7.71 (d, J = 8.5 Hz, 1H, H-7), 7.54 (t, J = 7.8 Hz, 1H, H-8), 3.80 (s, 3H, CH₃). EI-MS: m/z (I%) 201 (100, M⁺), 173 (50.2), 172 (42.7), 145 (15.8), 144 (88.2), 130 (30.1), 129 (32.4), 106 (11.5), 105 (21.2), 104 (55.5), 103 (38.1), 102 (84.3), 78 (29.4), 77 (90.7), 76 (71.8), 75 (45.1), 74 (45.1), 74 (17.0), 71 (15.3), 70 (10.6), 69 (20.0), 65 (10.3), 60 (26.6), 43 (44.2), 42 (23.1), 41 (28.7), 40 (30.9). LC-MS: m/z 202 [M+H]+. Anal. Calcd. for C₁₆H₁₁N₅O₂: C, 53.10; H, 2.67; N, 37.15. Found: C, 53.14; H, 2.65; N, 37.18.

2-(5-Oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetonitrile (2.5)
Yield 88.4%; mp 230–235 °C; IR (cm⁻¹): 3117, 3075, 3006, 2917, 2849, 1729, 1620, 1588, 1556, 1485, 1463, 1422, 1400, 1359, 1350, 1317, 1297, 1263, 1239, 1200, 1173, 1107, 1094, 1057, 1024, 1010, 983, 959, 920, 855, 779, 756, 734, 712, 686, 673. ¹H NMR: δ (ppm) 8.43 (d, J = 7.7 Hz, 1H, H-10), 7.95 (t, J = 7.9 Hz, 1H, H-9), 7.85 (d, J = 8.5 Hz, 1H, H-7), 7.61 (t, J = 7.4 Hz, 1H, H-8), 5.54 (s, 2H, CH₂). LC-MS: m/z 227 [M+H]+. Anal. Calcd. for C₁₆H₁₁N₅O₂: C, 53.10; H, 2.67; N, 37.15. Found: C, 53.14; H, 2.65; N, 37.18.

6-(2-Oxoo-2-phenethyl)tetrazolo[1,5-c]quinazolin-5(6H)-one (2.6)
Yield 95.6%; mp 199–201 °C; IR (cm⁻¹): 2919, 2850, 1747, 1696, 1623, 1593, 1557, 1417, 1465, 1449, 1400, 1377, 1340, 1296, 1259, 1227, 1196, 1174, 1130, 1111, 1098, 1076, 1055, 1026, 997, 970, 835, 812, 750, 728, 708, 687, 670, 632. ¹H NMR: δ (ppm) 8.46 (d, J = 7.6 Hz, 1H, H-10), 8.20 (d, J = 7.5 Hz, 2H, Ph-2,6), 7.78 (t, J = 7.8 Hz, 1H, H-9), 7.72 (d, J = 6.9 Hz, 1H, H-7), 7.59 (m, 4H, Ph-3,4,5, H-8), 6.04 (s, 2H, NCH₂). EI-MS: m/z (% rel.) 305 (26.6, M⁺), 277 (43.7), 207 (13.4), 129 (33.2), 118 (21.2), 117 (20.4), 116 (23.3), 92 (18.4), 91 (14.8), 90 (100), 89 (51.2), 88 (11.9), 65 (29.4), 64 (25.7), 63 (48.0), 62 (28.5), 57 (23.8), 55 (11.7), 52 (17.6), 51 (54.9), 50 (16.2). LC-MS: m/z 306 [M+H]+. Anal. Calcd. for C₁₆H₁₁N₅O₂: C, 62.95; H, 3.63; N, 22.94. Found: C, 62.99; H, 3.60; N, 22.97.

6-(2-Oxo-2-(p-tolyl)ethyl)tetrazolo[1,5-c]quinazolin-5(6H)-one (2.7)
Yield 64.5%; mp 220–222 °C; IR (cm⁻¹): 3045, 2997, 2957, 2916, 2847, 1732, 1684, 1621, 1604, 1589, 1557, 1530, 1488, 1470, 1432, 1354, 1318, 1295, 1264, 1233, 1202, 1185, 1124, 1106, 1091, 1057, 1035, 998, 972, 887, 879, 870, 861, 839, 829, 813, 782, 774, 753, 731, 704, 670, 654, 624. ¹H NMR: δ (ppm) 8.46 (d, J = 7.5 Hz, 1H, H-10), 8.10 (d, J = 6.8 Hz, 2H, Ph-2, 6), 7.80 (t, J = 7.4 Hz, 1H, H-9), 7.58 (d, J = 7.8 Hz, 2H, H-7, 8), 7.43 (d, J = 7.1 Hz, 2H, Ph-3, 5), 6.01 (s, 2H, NCH₂), 3.17 (s, 3H, CH₃). LC-MS: m/z 320 [M+H]+. Anal. Calcd. for C₁₆H₁₃N₅O₂: C, 63.94; H, 4.10; N, 21.93. Found: C, 63.90; H, 4.16; N, 21.88.

Methyl 2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetate (3.1)
Yield 14.5%; mp 194–196 °C; IR (cm⁻¹): 2953, 2918, 2851, 1718, 1619, 1589, 1580, 1556, 1487, 1455, 1400, 1371, 1351, 1324, 1295, 1259, 1227, 1188, 1171, 1161, 1107, 1096, 1050, 1005, 974, 960, 853, 807, 785, 756.
Ethyl 2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetate (3.2)

Yield 54.4%; mp 172–174 °C; IR (cm⁻¹): 2984, 2918, 1730, 1621, 1588, 1458, 1467, 1444, 1428, 1377, 1354, 1297, 1268, 1223, 1202, 1107, 1093, 1057, 1032, 1018, 990, 958, 887, 854, 814, 772, 752, 726, 707, 683, 672, 647. ¹H NMR: δ (ppm) 8.44 (d, J = 7.7 Hz, 1H, H-10), 7.88 (t, J = 7.8 Hz, 1H, H-9), 7.67 (d, J = 8.5 Hz, 1H, H-7), 7.59 (t, J = 7.2 Hz, 1H, H-8), 5.22 (s, 2H, NCH₂), 4.27 (dd, J = 13.1, 6.2 Hz, 2H, OCH₂), 1.32 (t, J = 6.9 Hz, 2H, CH₃). ¹³C NMR: δ 167.38 (C₂O), 150.01 (s, C-5), 142.93 (s, C-6a), 137.83 (s, C-1a), 134.68 (s, C-8), 125.68 (s, C-9), 125.09 (s, C-10), 116.20 (s, C-9), 108.19 (s, C-10a), 61.75 (s, OCH₃), 45.59 (s, NCH₃), 14.07 (s, CH₃). LC-MS: m/z 275 [M+H]+. Anal. Calcd. for C₁₁H₉N₅O₃; C, 52.75; H, 4.06; N, 25.63. Found: C, 52.71; H, 4.09; N, 25.60.

Propyl 2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetate (3.3)

Yield 72.5%; mp 108–110 °C; IR (cm⁻¹): 3128, 3060, 3019, 2959, 2918, 2873, 2849, 1730, 1620, 1589, 1557, 1519, 1487, 1466, 1426, 1395, 1351, 1298, 1267, 1220, 1199, 1106, 1093, 1058, 1029, 1010, 991, 958, 934, 876, 841, 827, 749, 707, 671, 648. ¹H NMR: δ (ppm) 8.44 (d, J = 7.6 Hz, 1H, H-10), 7.85 (t, J = 7.7 Hz, 1H, H-9), 7.62 (d, J = 8.5 Hz, 1H, H-7), 7.57 (t, J = 7.4 Hz, 1H, H-8), 5.20 (s, 2H, NCH₂), 4.19 (t, J = 6.5 Hz, 2H, OCH₂), 1.38 (d, J = 14.7, 7.3 Hz, 2H, OCH₂CH₂CH₂), 0.93 (t, J = 7.2 Hz, 3H, OCH₂CH₂CH₂). Anal. Calcd. for C₁₃H₁₅N₅O₃; C, 45.35; H, 4.56; N, 24.38. Found: C, 45.37; H, 4.54; N, 24.41.

N-(2-Methoxyphenyl)-N-(2-(2-methoxyphenyl)amino)-2-oxoethyl-2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetamide (5.1)

Yield 72.2%; mp 184–186 °C; IR (cm⁻¹): 3234, 3004, 2953, 2919, 2850, 1771, 1710, 1683, 1600, 1539, 1486, 1453, 1417, 1379, 1360, 1321, 1290, 1264, 1253, 1237, 1199, 1179, 1159, 1080, 1037, 996, 970, 950, 907, 864, 841, 827, 815, 799, 784, 747, 723, 698, 682, 668, 644, 621. ¹H NMR: δ (ppm) 10.42 (s, 1H, NH), 8.26 (d, J = 7.0 Hz, 1H, H-10), 7.64 (dd, J = 13.1, 5.0 Hz, 3H, H-7,8,9), 7.32–7.23 (m, 2H, Ph'-3, Ph-3), 7.17 (t, J = 8.1 Hz, 1H, Ph'-4), 7.05 (d, J = 7.8 Hz, 1H, Ph'-6), 6.97–6.83 (m, 3H, Ph'-5, Ph-5, Ph-6), 5.66 (s, 2H, NCH₂CO), 4.46 (s, 2H, NCH₂CONH), 3.77 (s, 1H, Ph'-OCH₂), 3.75 (s, 1H, Ph-OCH₂). EI-MS: m/z (% rel) 513 (33.0, M⁺), 335 (51.9), 309 (10.9), 308 (32.7), 307 (26.3), 178 (17.9), 150 (34.0), 149 (100), 148 (17.2), 147 (15.5), 123 (10.1), 119 (19.9), 92 (12.9), 91 (18.9), 89 (10.2), 86 (39.6), 84 (41.2), 57 (18.1), 55 (10.7). LC-MS: m/z 513 [M+H]⁺. Anal. Calcd. for C₂₀H₁₇F₁N₂O₃; C, 60.81; H, 4.51; N, 19.09. Found: C, 60.85; H, 4.47; N, 19.12.
N-(2-Oxo-2-((4-(trifluoromethyl)benzylo)amino)ethyl)-2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)-N-(4-(trifluoromethyl)benzylo)acetamide (5.4)
Yield 31.1%; mp 166–168 °C; IR (cm⁻¹): 3293, 2916, 1774, 1708, 1671, 1621, 1558, 1486, 1452, 1439, 1422, 1412, 1374, 1234, 1260, 1203, 1178, 1158, 1114, 1080, 1067, 1044, 1019, 953, 925, 817, 784, 762, 744, 735, 712, 679, 635, 613. 1H NMR: δ (ppm) 8.99 (brs, 1H, NH), 8.20 (d, J = 6.8 Hz, 1H, H-10), 7.63 (m, 7H, H-7,8,9, Ph-1,2,3,5,6), 5.52 (m, 2H, NCH₂), 3.63 (s, 2H, H-2), 3.48 (s, 2H, H-6). LC-MS: m/z 590 [M+H]+. Anal. Calcd. for C₂₄H₁₈F₃N₇O₃: C, 58.96; H, 4.45; N, 26.74.

6-(2-Morpholino-2-oxoethyl)tetrazolo[1,5-c]quinazolin-5(6H)-one (6.1)
Yield 73.3%; mp 217–219 °C; IR (cm⁻¹): 3291, 2917, 2868, 2848, 1733, 1665, 1620, 1587, 1556, 1485, 1454, 1422, 1399, 1365, 1344, 1329, 1314, 1299, 1269, 1261, 1226, 1213, 1198, 1163, 1120, 1010, 1006, 1037, 1024, 1008, 987, 949, 908, 872, 842, 801, 780, 760, 730, 709, 669, 621. 1H NMR: δ (ppm) 8.43 (d, J = 7.5 Hz, 1H, H-10), 7.91 (t, J = 7.5 Hz, 1H, H-9), 7.63 (d, J = 8.7 Hz, 1H, H-7), 7.59 (t, J = 7.4 Hz, 1H, H-8), 5.38 (s, 2H, NCH₂), 3.75 (s, 2H, H-3 morph), 3.71 (s, 3H, 1H, H-5 morph), 3.63 (s, 2H, H-2 morph), 3.48 (s, 2H, H-6 morph). EI-MS: m/z 315 [M]+. Anal. Calcd. for C₁₄H₁₄N₆O₃: C, 53.50; H, 4.49; N, 26.74.

6-(2-(4-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)tetrazolo[1,5-c]quinazolin-5(6H)-one (6.2)
Yield 96.8%; mp 247–249 °C; IR (cm⁻¹): 3295, 2963, 2917, 2849, 1727, 1656, 1618, 1589, 1557, 1504, 1485, 1463, 1447, 1435, 1397, 1377, 1359, 1341, 1239, 1294, 1279, 1262, 1235, 1213, 1199, 1166, 1149, 1106, 1091, 1057, 1039, 1025, 1009, 994, 960, 937, 927, 803, 789, 755, 730, 670, 616. 1H NMR: δ (ppm) 8.44 (d, J = 7.8 Hz, 1H, H-10), 7.91 (t, J = 7.9 Hz, 1H, H-9), 7.65 (d, J = 8.4 Hz, 1H, H-7), 7.60 (t, J = 7.3 Hz, 1H, H-8), 7.21–7.15 (m, 2H, Ph-5,6), 7.12 (t, J = 8.2 Hz, 1H, Ph-4), 7.04 (dd, dd, J = 12.3, 5.9 Hz, 1H, Ph-3), 5.43 (s, 2H, NCH₂), 3.87 (s, 2H, ppz-3), 3.67 (s, 2H, ppz-5), 3.21 (s, 2H, ppz-2), 3.05 (s, 2H, ppz-6). 13C NMR: δ (ppm) 164.20 (s, CO ppz), 150.38 (s, CF), 143.30 (s, C-5), 140.09 (s, Ph-1), 138.74 (s, C-6), 135.00 (s, C-1a), 125.90 (s, C-9), 125.38 (s, Ph-5), 125.24 (s, C-8), 123.49 (s, C-7), 120.14 (s, C-10), 119.95 (s, Ph-6), 116.65 (s, Ph-4), 116.48 (s, Ph-3), 108.42 (s, C-10a), 50.97 (s, C-3 ppz), 50.49 (s, C-5 ppz), 46.02 (s, NCH₂), 44.97 (s, C-2), 42.31 (s, C6 ppz). LC-MS: m/z 408 [M+H]+. Anal. Calcd. for C₁₄H₁₄F₂N₄O₂: C, 58.96; H, 4.45; N, 24.07. Found: C, 58.98; H, 4.42; N, 24.09.

3. Results and Discussion

3.1. Chemistry

The tetrazolo[1,5-c]quinazoline synthesis was described in detail in our previous works. 11,12 5-(2'-Aminophenyl)-1H-pyrazol-1-yl)ethylyl)tetrazolo[1,5-c]quinazolin-5(6H)-one (1.1), which was used as the starting compound for further modifications at position 6. 

The tetrazolo[1,5-c]quinazolin-5(6H)-one (1.1) was dissolved in DMSO with an equimolar amount of sodium hydride. The corresponding halogen derivative was added only after all hydrogen has been released. The resulting mixture was refluxed for 2 h. Alternatively, the reaction was performed by the addition of potassium carbonate in DMSO or sodium bicarbonate in dioxane. The best yields and purity of derivatives 2 were observed in the presence of sodium hydride. This method was chosen as the primary one.
The next step was the synthesis of acetamides. Firstly 2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetic acid should be obtained with further aminolysis. The direct alkylation of tetrazolo[1,5-c]quinazolin-5(6H)-one (1.1) with chloroacetic acid has not resulted in the desirable product. Thus, an alkaline hydrolysis of 2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetate esters 3.1–3.3 was necessary (Scheme 1). However, the cleavage of quinazoline cycle was observed, and the product of the cleavage turned out to be 2-((2-(1H-tetrazol-5-yl)phenyl)amino)acetic acid (4.1) (see further discussion on spectral data).

Then, N-alkylation of tetrazolo[1,5-c]quinazolin-5(6H)-one (1.1) with chloracetamides was used to synthesize amides 5.1–5.4. The reaction was quite interesting, since the products obtained were disubstituted compounds 5.1–5.4. In this reaction NH proton of acetamide was acting as a competitive acid moiety, which results in the alkylation of the quinazolin-5(6H)-one NH group, and of acetamide NH group of intermediate alkylated product (Scheme 2). This was confirmed by LC-MS and 1H NMR spectra of the synthesized compounds with intensive peaks of molecular ions with a mass of two acetamide residues. Alkylation with tertiary amides 6.1–6.4 has not revealed any unexpected products (Scheme 2).

The identity of the synthesized compounds was confirmed by IR, LC-, EI-MS, 1H, 13C NMR, and elemental analysis. LC-MS of the synthesized compounds in a »soft« ionization (chemical ionization at atmospheric pressure) allowed to register the molecular ion peak [M+1] in high intensity. For compound 4.1 the ion with molecular weight of 220 was observed, confirming cleavage of the quinazoline ring.

In the 1H NMR spectra of tetrazolo[1,5-c]quinazolin-5(6H)-ones the clear splitting of aromatic quinazoline protons’ signals was observed. Thus, H-10 signal can be found at a range of 8.19–8.46 ppm, H-9 at 7.71–7.95 ppm, H-7 at 7.50–7.85 ppm, and H-8 at 7.42–7.61 ppm. The signals for these protons for some compounds were overlapping with each other (2.3, 2.7) or with other aromatic substituents protons (5.4). At the same time, for compound 4.1 the diamagnetic shift of aromatic protons was observed, obviously due to the absence of electron-deficient tetrazoloquinazoline system. Thus, Ph-3 signal was registered at 7.80 ppm as a doublet, Ph-4 at 7.30 ppm as a triplet, Ph-5 at 6.72 ppm as a triplet, and Ph-6 as a doublet at 6.66 ppm. Besides that, the NH tetrazole proton was detected as a broad singlet at 8.22–7.93 ppm. The signal of the NCH2 group can be used as a confirmation of N-alkylation. For compounds 2.1–2.7 it was registered as a two-proton singlet at 5.54–3.80 ppm, except for compound 2.4, where it was as a two-proton triplet at 4.55 ppm. Due to electron acceptor influence of 2-oxo-2-phenylethyl and 2-oxo-2-(p-tolyl)ethyl moiety the signal of NCH3 group was observed in the weak field at 6.04–6.01 ppm for substances 2.6 and 2.7. Esters 3.1–3.3 displayed two-proton singlet signal at 5.20–5.22 ppm. For 2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetic acid the signal of NCH3 group was detected in the stronger field at 4.01 ppm. Moreover, for acetamides 5.1–5.4 and...
tertiary amides 6.1–6.4 the signal of NCH$_3$ group was also shifted to the weak field and observed at 5.82–5.38 ppm as a two-proton singlet. Only for compound 6.3 the NCH$_3$ signal was overlapped with H-5 of pyrazol and registered as a multiplet at 5.76–5.51 ppm. The signal of NH proton of phenylacetamides 5.1 and 5.2 was located as a singlet at 10.42–10.95 ppm, whereas for benzylacetamides 5.3 and 5.4 as an unsplitted triplet at 8.86–8.99 ppm. All alkyl groups are located in the strong field.

As for the IR spectra, the main tetrazolo[1,5-c]quinazoline ring and C–H deformations were detected at 1623–1485 cm$^{-1}$ and at 917–608 cm$^{-1}$. Azo fragments had stretchings at 1604–1400 cm$^{-1}$. Moderate absorptions caused by cyclic N–C(=O)–N stretching were overlapped with ester and amide carbonyl vibrations. Vibrations of the ν$_{C=O}$ in esters 3.1–3.3 were found at 1746–1730 cm$^{-1}$. Wide stretchings of C–O–C appeared at 1250–1188 cm$^{-1}$. The carbonyl stretchings were overlapped with H-5 and observed at 5.82–5.38 ppm in esters 3.1–3.3. The signal of NH proton of phenylacetamides 5.1 and 5.2 was located as a singlet at 10.42–10.95 ppm, whereas for benzylacetamides 5.3 and 5.4 as an unsplitted triplet at 8.86–8.99 ppm. All alkyl groups are located in the strong field.

3.2. Anticancer Assay for Preliminary in vitro Testing

Among all newly synthesized compounds substances 2.7, 3.2, 5.2, 5.3 were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program for

| Cmpd. | Mean | Range |
|-------|------|-------|
| 2.7   | 103.29 | 81.02–127.01 |
| 3.2   | 101.85 | 85.49–118.12 |
| 5.2   | 102.25 | 84.19–128.99 |
| 5.3   | 95.34 | 65.64–115.96 |

*a L – leukemia, nscLC – non-small cell lung cancer, CoC – colon cancer, CNSC – CNS cancer, M – melanoma, OV – ovarian cancer, RC – renal cancer, PC – prostate cancer, BC – breast cancer, bold values – the most sensitive ones.

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the in vitro cell line screening to investigate their anticancer activity.\textsuperscript{15,16} (Table 1).

The most sensitive cell line turned out to be UO-31 of renal cancer. It should be mentioned that N-(4-fluorobenzyl)-N-(2-(4-fluorobenzyl)amino)-2-oxoethyl)-2-(5-oxotetrazolo[1,5-c]quinazolin-6(5H)-yl)acetamide (5.3) had the highest inhibition at 33.55%. Besides, this compound also negatively influenced RPMI-8226 of leukemia cell line, displaying inhibition at 34.36%.

4. Conclusions

Due to their unique characteristics, compounds with tetrazole ring have been used in pharmaceutical and clinical applications especially as anticancer agents. In this work, novel 6-N-R-tetrazolo[1,5-c]quinazolin-5(6H)-ones were synthesized. N-Alkylation reaction of tetrazolo[1,5-c]quinazolin-5(6H)-one (1.1) with various chloro-derivatives was performed under various reaction conditions. As the best option a reflux in DMF with an equimolar amount of sodium hydride was selected. Spectral data confirm the best option a reflux in DMF with an equimolar amount of sodium hydride was selected. Spectral data confirm molecular structures of investigated compounds. These investigations will be continued for other activities and core structures.

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Povzetek

Spojine, ki vsebujejo tetrazolski obroč, so zelo animivši sistemi s pomembnimi farmacevtskimi in kliničnimi uporabami, zlasti kot učinkovine proti raku. V tem članku predstavljamo sinteze novih 6-N-R-tetrazolo[1,5-c]quinazolin-5(6H)-onov, katerih strukturo in čistost smo ugotovili s pomočjo zbranih IR, LC-, EI-MS, ¹H in ¹³C NMR podatkov ter rezultatov elementnih analiz. Opisujemo podrobnosti sinteze, torej N-alkiliranja, vključno z reakcijami s sekundarnimi in terciarnimi amidi. Štiri nove pripravljene spojine (2.7, 3.2, 5.2, 5.3) smo in vitro testirali za protirakavo učinkovitost pri 10 µM na 60 cellnih linij devetih vrst rakov: levkemije in melanoma ter raka pljuč, dobelega črevjesja, centralnega živčnega sistema, jajčnika, ledvice, prostate in dojke. V prihodnostih bomo pozikišali izvesti še dodatne sinteze spojin iz serije tetrazolo[1,5-c]quinazolinskih sistemov z namenom izboljšanja protirakave učinkovitosti.