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RESEARCH ARTICLE

2-Alkyl(aryl)-quinazolin-4(3*H*)-thiones, 2-R-(quinazolin-4(3*H*)-ylthio)carboxylic acids and amides: synthesis, molecular docking, antimicrobial and anticancer properties

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Abstract

In this study, a series of novel 2-alkyl(aryl)-quinazolin-4(3*H*)-thiones, 2-R-(quinazolin-4(3*H*)-ylthio)carboxylic acids and amides were synthesized and evaluated for antimicrobial and anticancer activities. Their structure was confirmed by elemental analysis and spectral data (FT-IR, LC-MS, ^{1}H-NMR). Antimicrobial activity was tested *in vitro* against *Staphylococcus aureus, Enterococcus faecalis*, *Enterobacter aerogenes, Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans* and NCI *in vitro* preliminary anticancer activity against nine different cancer types. The most active antibacterial and antifungal compounds were: **2.1**, **2.2** and **2.4**. The introduction of the carboxylic acid or amide residue into the fourth position of quinazolin-4(3*H*)-thione resulted in the absence of antimicrobial activity. Substance **3.8** inhibited renal cancer UO-31 line and **2.18** – leukemia CCRF-CEM. The results of *in silico* molecular docking for DHFR and CK2 kinase had no correlation with *in vitro* properties, proposing the presence of other biological activity pathways.

Keywords

2-Alkyl(aryl)-quinazolin-4(3*H*)-thiones, antibiotic, anticancer, antifungal, molecular docking

History

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Introduction

According to literature data, substituted quinazolines, i.e. condensed benzene–pyrimidine system, are intensively investigated for biological activities during several decades. In this paper, quinazoline-4(3*H*)-thione was chosen as the main structural fragment, as its derivatives have proven to show a broad spectrum of biological effects, such as anticonvulsant[1], antidiabetic[2], insecticidal[3], cardiotonic with myofibrillar Ca^{2+} sensitizing effect[4], inhibition of thymidylate synthase[5], of tyrosine kinase domain of c-Src Ple[6] and of phosphodiesterase[7].

Given the constant and increasing threat of drug resistance, we were particularly interested in antimicrobial properties of quinazoline-4(3*H*)-thione's derivatives. It was found that 4-(*S*-butylthio)quinazoline was more active than isoniazide, against atypical strains of mycobacteria, when investigated for antitubercular activity against *Mycobacterium tuberculosis*, *M. kansasii*, *M. fortuitum*, *M. avium* and *M. intracellulare*[8]. The 4-ethylthio-6-fluoroquinazoline had potent antifungal activities against practically all the tested fungi (*Gibberella zeae*, *Fusarium oxysporum*, *Cardamine mandshurica*, *Rhizoctonia solani*, *Thanatephorus cucumeris*, *Phytophthora infestans*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*) and showed a broad-spectrum bioactivity[9]. Also quinazoline-4(3*H*)-thione's derivatives possessed antifungal properties against *Sclerotium cepivorum* and *Botrytis allii*[10]. Even bis-quinazolines showed antimicrobial activity[11].

Taking into consideration the above facts, we aimed to synthesize a series of novel 2-R-quinazolin-4(3*H*)-thiones in order to obtain the compounds with high antimicrobial and antifungal activity. Modification of thion pharmacophore with carboxyl or amide residue could influence the manifestation of pharmacological action of the synthesized compounds; especially since the 7-oxo-2-(trifluoromethyl)-4,7-dihydro-[1,2,4]triazolo[5,1-*a*]pyrimidine-6-carboxylic acids were reported to inhibit the growth of *M. tuberculosis* to 92%[12]. Substituted 1-ethyl-6-fluorine-hydro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid had better antimicrobial properties against *Xanthomonas oryzae*, than norfloxacin and against *Xanthomonas axonopodis*, *Erwinia aroideae* and *Rhizoctonia solan*, than streptomycin sulfate[13]. It was found that 2-(2-(4-(trifluoromethyl)benzylidene)hydrazinyl)-*N*-(4-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)-phenyl)acetamide was the most active compound in its series, when investigated for analgesic, anti-inflammatory and *in vitro* antimicrobial activities[14]. Moreover, it is known that quinazoline derivatives are used in medicine as an effective anticancer agents[15,16]. For example, Afatinib is a tyrosine kinase inhibitor (TKI) that also irreversibly inhibits human epidermal growth factor receptors (HER2 and HER4) and epidermal growth factor receptor (EGFR)[17].

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Hence, the synthesis of (2-R-quinazolin-4(3*H*)-ylthio)carboxylic acids and amides was of undoubted interest from a synthetic and biological activity point of view.

Experimental

Chemistry

General

Melting points were determined in open capillary tubes in a Thiele's apparatus and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL cube analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module ATR eco ZnSe. ^{1}H NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 spectrometer (Varian, Palo Alto, CA) 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 ''Agi1ent 1100 Series'' (Agilent, Palo Alto, CA) equipped with diode-matrix and mass-selective detector ''Agi1ent LC/MSD SL'' (atmospheric pressure chemical ionization – APCI). The purity of all obtained compounds was checked by ^{1}H NMR and LC-MS.

2-R-4(3*H*)-Quinazolone derivatives were obtained as reported in literature[18]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

Materials and methods

*Preparation of 2-alkyl(aryl)-quinazolin-4-thiones (**2.1–2.18**).* 2-Alkyl(aryl)-quinazolin-4(3*H*)-one (5 mmol) was added to the solution of 2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (5 mmol, Lawesson's reagent) in dioxane (20 ml) and refluxed for 1–2 h. The mixture was poured into the cold water. The precipitate was filtered and dried. Substances were recrystallized from a mixture of dioxane–water (2:1) (**2.7**, **2.9**), 2-propanol–water (1:3) (**2.4**, **2.10**), DMF–water (**2.8**, **2.12–2.14**, **2.16**, **2.17**), acetone (**2.5**, **2.11**), acetic acid (**2.3**, **2.6**, **2.15**, **2.18**) or reprecipitated (**2.2**).

*3H-Quinazoline-4-thione (**2.1**).* Yellow solid. Yield: 90.0%, m.p. 302–304 °C. IR (cm^{-1}): 3039, 3010, 2982, 2830, 2792, 2735, 2699, 2662, 2626, 2489, 1815, 1685, 1621, 1595, 1566, 1517, 1463, 1441, 1396, 1341, 1302, 1261, 1243, 1197, 1107, 1026, 987, 954, 906, 864, 809, 771, 760, 682, 669, 639. ^{1}H NMR: δ (ppm) 13.86 (br s, 1H, NH), 8.59 (d, $J = 8.0$ Hz, 1H, H^{5}q.), 8.19 (s, 1H, H^{2}q.), 7.90 (t, $J = 7.5$ Hz, 1H, H^{7}q.), 7.74 (d, $J = 8.0$ Hz, 1H, H^{8}q.), 7.62 (t, $J = 7.1$ Hz, 1H, H^{6}q.). LC-MS: $m/z = 163$ $[M + H]^+$. Anal. Calcd. for $C_8H_6N_2S$ C, 59.24; H, 3.73; N, 17.27; S, 19.77. Found: C, 59.22; H, 3.75; N, 17.26; S, 19.78.

*2-Methyl-3H-quinazoline-4-thione (**2.2**).* Yellow solid. Yield: 73.9%, m.p. 218–220 °C. IR (cm^{-1}): 3167, 3116, 3029, 2977, 2899, 2862, 2784, 2691, 2649, 1667, 1607, 1568, 1504, 1467, 1454, 1419, 1378, 1342, 1292, 1251, 1234, 1213, 1201, 1164, 1142, 1111, 1098, 1040, 1025, 999, 964, 880, 870, 805, 774, 762, 692, 660, 642. ^{1}H NMR: δ (ppm) 13.72 (br s, 1H, NH), 8.55 (d, $J = 8.1$ Hz, 1H, H^{5}q.), 7.87 (t, $J = 7.6$ Hz, 1H, H^{7}q.), 7.64 (d, $J = 8.0$ Hz, 1H, H^{8}q.), 7.54 (t, $J = 7.6$ Hz, 1H, H^{6}q.), 2.48 (s, 3H, CH_3). LC-MS: $m/z = 177$ $[M + H]^+$. Anal. Calcd. for $C_9H_8N_2S$ C, 61.34; H, 4.58; N, 15.89; S, 18.19. Found: C, 61.38; H, 4.60; N, 15.84; S, 18.18.

*2-Ethyl-3H-quinazoline-4-thione (**2.3**).* Yellow solid. Yield: 77.2%, m.p. 185–187 °C. IR (cm^{-1}): 3187, 3138, 3094, 3073, 3042, 2979, 2918, 2875, 2848, 2729, 2693, 2660, 2629, 2598, 2568, 2508, 1959, 1855, 1770, 1743, 1714, 1660, 1617, 1603, 1566, 1505, 1464, 1452, 1417, 1371, 1343, 1312, 1259, 1227, 1185, 1148, 1124, 1076, 1034, 1013, 971, 917, 886, 854, 837, 802, 768, 642. ^{1}H NMR: δ (ppm) 13.74 (br s, 1H, NH), 8.56 (d, $J = 8.1$ Hz, 1H, H^{5}q.), 7.87 (t, $J = 7.6$ Hz, 1H, H^{7}q.), 7.67 (d, $J = 8.1$ Hz, 1H, H^{8}q.), 7.55 (t, $J = 7.6$ Hz, 1H, H^{6}q.), 2.77 (q, $J = 7.5$ Hz, 2H, CH_2CH_3), 1.27 (t, $J = 7.5$ Hz, 3H, CH_2CH_3). LC-MS: $m/z = 191$ $[M + H]^+$. Anal. Calcd. for $C_{10}H_{10}N_2S$ C, 63.13; H, 5.30; N, 14.72; S, 16.85. Found: C, 63.12; H, 5.34; N, 14.73; S, 16.82.

*2-Phenyl-3H-quinazoline-4-thione (**2.4**).* Yellow solid. Yield: 53.6%, m.p. 200–202 °C. IR (cm^{-1}): 3140, 3115, 3029, 2975, 2905, 1662, 1599, 1565, 1492, 1463, 1447, 1429, 1345, 1316, 1299, 1284, 1252, 1241, 1220, 1145, 1132, 1080, 1029, 959, 945, 928, 889, 857, 808, 758, 701, 689, 658, 614. ^{1}H NMR: δ (ppm) 13.93 (br s, 1H, NH), 8.64 (d, $J = 7.6$ Hz, 1H, H^{5}q.), 8.20 (d, $J = 7.5$ Hz, 2H, H^2, H^{6}ph.), 7.93 (t, $J = 8.0$ Hz, 1H, H^{7}q.), 7.79 (d, $J = 7.5$ Hz, 1H, H^{8}q.), 7.67–7.54 (m, 4H, H^{6}q., H$^{3–5}$ph.). LC-MS: $m/z = 239$ $[M + H]^+$. Anal. Calcd. for $C_{14}H_{10}N_2S$ C, 70.56; H, 4.23; N, 11.75; S, 13.45. Found: C, 70.54; H, 4.23; N, 11.74; S, 13.43.

*2-Benzyl-3H-quinazoline-4-thione (**2.5**).* Orange solid. Yield: 64.0%, m.p. 200–202 °C. IR (cm^{-1}): 3139, 3117, 3087, 3062, 3031, 2969, 2915, 2892, 2875, 2497, 1674, 1651, 1618, 1598, 1572, 1507, 1494, 1464, 1454, 1435, 1414, 1342, 1318, 1254, 1232, 1181, 1155, 1122, 1072, 1028, 958, 919, 893, 865, 838, 827, 763, 729, 717, 695, 667, 658, 642, 610. ^{1}H NMR: δ (ppm) 13.98 (br s, 1H, NH), 8.56 (d, $J = 8.1$ Hz, 1H, H^{5}q.), 7.87 (t, $J = 7.5$ Hz, 1H, H^{7}q.), 7.68 (d, $J = 8.1$ Hz, 1H, H^{8}q.), 7.57 (t, $J = 7.6$ Hz, 1H, H^{6}q.), 7.41 (d, $J = 7.4$ Hz, 2H, H^2, H^{6}ph.), 7.35 (t, $J = 7.4$ Hz, 2H, H^3, H^{5}ph.), 7.27 (t, $J = 7.0$ Hz, 1H, H^{4}ph.), 4.11 (s, 2H, CH_2). LC-MS: $m/z = 253$ $[M + H]^+$. Anal. Calcd. for $C_{15}H_{12}N_2S$ C, 71.40; H, 4.79; N, 11.10; S, 12.71. Found: C, 71.42; H, 4.80; N, 12.69; S, 11.12.

*2-Phenethyl-3H-quinazoline-4-thione (**2.6**).* Yellow solid. Yield: 89.0%, m.p. 185–187 °C. IR (cm^{-1}): 3138, 3079, 3026, 2971, 2919, 2723, 2502, 1977, 1955, 1938, 1850, 1738, 1714, 1654, 1603, 1567, 1505, 1494, 1464, 1449, 1430, 1346, 1322, 1270, 1231, 1212, 1146, 1073, 1031, 1020, 994, 969, 955, 902, 847, 766, 737, 691, 645, 619. ^{1}H NMR: δ (ppm) 13.82 (br s, 1H, NH), 8.57 (d, $J = 8.1$ Hz, 1H, H^{5}q.), 7.88 (t, $J = 7.6$ Hz, 1H, H^{7}q.), 7.70 (d, $J = 8.1$ Hz, 1H, H^{8}q.), 7.57 (t, $J = 7.6$ Hz, 1H, H^{6}q.), 7.31 (d, $J = 5.0$ Hz, 4H, H^2, H^3, H^5, H^{6}ph), 7.21 (t, $J = 5.1$ Hz, 1H, H^{4}ph.), 3.13–3.01 (m, 4H, CH_2CH_2). LC-MS: $m/z = 267$ $[M + H]^+$. Anal. Calcd. for $C_{16}H_{14}N_2S$ C, 72.15; H, 5.30; N, 10.52; S, 12.04. Found: C, 72.37; H, 5.28; N, 10.48; S, 12.01.

*2-Styryl-3H-quinazoline-4-thione (**2.7**).* Yellow solid. Yield: 68.2%, m.p. 246–248 °C. IR (cm^{-1}): 3158, 3100, 3025, 2966, 2917, 2849, 2505, 1739, 1666, 1643, 1608, 1568, 1503, 1469, 1446, 1434, 1346, 1328, 1255, 1222, 1192, 1161, 1148, 1069, 1033, 1016, 981, 962, 871, 842, 830, 779, 766, 747, 690, 676, 604. ^{1}H NMR: δ (ppm) 13.83 (br s, 1H, NH), 8.58 (d, $J = 8.0$ Hz, 1H, H^{5}q.), 8.05 (d, $J = 6.1$ Hz, 1H, HetCH=), 7.90 (t, $J = 7.5$ Hz, 1H, H^{7}q.), 7.76 (d, $J = 8.2$ Hz, 1H, H^{8}q.), 7.70 (d, $J = 7.6$ Hz, 2H, H^2, H^{6}ph.), 7.58 (t, $J = 7.6$ Hz, 1H, H^{6}q.), 7.54–7.42 (m, 3H, H$^{3–5}$ph.), 7.29 (d, $J = 6.0$ Hz, 1H, =CHC_6H_5). LC-MS: $m/z = 219$ $[M + H]^+$. Anal. Calcd. for $C_{16}H_{12}N_2S$ C, 72.70; H, 4.58; N, 10.60; S, 12.13. Found: C, 72.69; H, 4.56; N, 10.64; S, 12.17.

*2-Trifluoromethyl-3H-quinazoline-4-thione (**2.8**).* Yellow solid. Yield: 96.2%, m.p. 210–212 °C. IR (cm^{-1}): 3152, 3112, 3085,

3056, 2983, 2922, 1668, 1631, 1596, 1573, 1516, 1464, 1360, 1317, 1218, 1206, 1161, 1130, 1034, 899, 846, 800, 765, 692, 624. ^{1}H NMR: δ (ppm) 12.44 (br s, 1H, NH), 8.63 (d, $J = 8.0$ Hz, 1H, H^{5}q.), 7.99 (t, 1H, $J = 6.7$ Hz, H^{7}q.), 7.86 (d, $J = 8.0$ Hz, 1H, H^{8}q.), 7.76 (t, $J = 6.9$ Hz, 1H, H^{6}q.). LC-MS: $m/z = 230$ $[M + H]^+$. Anal. Calcd. for $C_9H_5F_3N_2S$ C, 46.96; H, 2.19; N, 12.17; S, 13.93. Found: C, 47.01; H, 2.22; N, 12.14; S, 13.93.

2-Morpholin-4-yl-3H-quinazoline-4-thione (**2.9**). Yellow solid. Yield: 86.5%, m.p. 202–204 °C. IR (cm^{-1}): 3245, 3197, 3137, 3055, 2959, 2900, 2851, 2571, 1620, 1573, 1490, 1456, 1434, 1406, 1369, 1336, 1303, 1279, 1254, 1227, 1178, 1153, 1131, 1109, 1063, 1028, 1019, 1000, 982, 918, 873, 859, 779, 755, 732, 713, 693, 633. ^{1}H NMR: δ (ppm) 12.88 (br s, 1H, NH), 8.44 (d, $J = 7.6$ Hz, 1H, H^{5}q.), 7.67 (t, $J = 6.4$ Hz, 1H, H^{7}q.), 7.44 (d, $J = 8.1$ Hz, 1H, H^{8}q.), 7.26 (t, $J = 6.6$ Hz, 1H, H^{6}q.), 3.76–3.64 (m, 8H, morph.). LC-MS: $m/z = 247$ $[M + H]^+$. Anal. Calcd. for $C_{12}H_{13}N_3OS$ C, 58.28; H, 5.30; N, 16.99; S, 12.96. Found: C, 58.31; H, 5.28; N, 17.01; S, 12.99.

2-(Morpholin-4-yl-methyl)-3H-quinazoline-4-thione (**2.10**). Yellow solid. Yield: 58.5%, m.p. 180–182 °C. IR (cm^{-1}): 3120, 3082, 3050, 2981, 2965, 2949, 2916, 2848, 2818, 2767, 2693, 2509, 1598, 1568, 1497, 1464, 1448, 1424, 1352, 1335, 1306, 1294, 1270, 1226, 1197, 1164, 1141, 1111, 1076, 1034, 1011, 987, 949, 910, 892, 870, 856, 839, 808, 782, 764, 683, 651, 604. ^{1}H NMR: δ (ppm) 13.99–13.35 (br s, 1H, NH), 8.57 (d, $J = 8.0$ Hz, 1H, H^{5}q.), 7.87 (t, $J = 7.4$ Hz, 1H, H^{7}q.), 7.71 (d, $J = 8.0$ Hz, 1H, H^{8}q.), 7.58 (t, $J = 7.5$ Hz, 1H, H^{6}q.), 3.59 (s, 4H, N(CH$_2$)$_2$), 3.57 (s, 2H, CH$_2$), 2.60–2.53 (m, 4H, O(CH$_2$)$_2$). LC-MS: $m/z = 261$ $[M + H]^+$. Anal. Calcd. for $C_{13}H_{15}N_3OS$ C, 59.75; H, 5.79; N, 16.08; S, 12.27. Found: C, 59.72; H, 5.81; N, 16.07; S, 12.24.

2-(Thiophen-3-yl)-3H-quinazoline-4-thione (**2.11**). Yellow solid. Yield: 80.3%, m.p. 215–219 °C. IR (cm^{-1}): 3239, 3093, 3064, 2914, 2847, 1609, 1586, 1557, 1487, 1461, 1430, 1415, 1345, 1312, 1288, 1250, 1220, 1187, 1143, 1068, 1027, 1015, 968, 942, 884, 865, 843, 801, 765, 717, 686, 622. ^{1}H NMR: δ (ppm) 13.80 (br s, 1H, NH), 8.80 (s, 1H, H^{2}thioph.), 8.61 (d, $J = 7.7$ Hz, 1H, H^{5}q.), 7.96–7.86 (m, 2H, H^{5}thioph., H^{7}q.), 7.75 (d, $J = 8.0$ Hz, 2H, H^{4}thioph., H^{8}q.), 7.59 (t, $J = 7.1$ Hz, 1H, H^{6}q.). LC-MS: $m/z = 246$ $[M + H]^+$. Anal. Calcd. for $C_{12}H_8N_2S_2$ C, 58.99; H, 3.30; N, 11.46; S, 26.25. Found: C, 59.07; H, 3.26; N, 11.43; S, 26.22.

2-(1H-Imidazol-1-yl)-3H-quinazoline-4-thione (**2.12**). Yellow solid. Yield: 54.0%, m.p. 235–237 °C. IR (cm^{-1}): 3111, 3065, 2980, 2921, 2853, 2760, 2638, 1677, 1626, 1604, 1580, 1516, 1465, 1431, 1396, 1356, 1340, 1317, 1288, 1255, 1227, 1183, 1121, 1102, 1074, 1034, 958, 920, 875, 862, 831, 761, 734, 690, 665, 646, 627, 604. LC-MS: $m/z = 242$ $[M + H]^+$. Anal. Calcd. for $C_{11}H_8N_4S$ C, 57.88; H, 3.53; N, 24.54; S, 14.05. Found: C, 57.83; H, 3.52; N, 24.57; S, 14.00.

2-(2-Chlorophenyl)-3H-quinazoline-4-thione (**2.13**). Red solid. Yield: 65.1%, m.p. 145–146 °C. IR (cm^{-1}): 2501, 1597, 1585, 1557, 1533, 1467, 1446, 1425, 1319, 1288, 1268, 1238, 1198, 1152, 1059, 1018, 966, 946, 936, 910, 876, 860, 838, 767, 754, 723, 707, 650, 635. ^{1}H NMR: δ (ppm) 10.04 (br s, 1H, NH), 8.74 (d, $J = 8.2$ Hz, 1H, H^{5}q.), 8.10 (t, $J = 7.5$ Hz, 1H, H^{7}q.), 7.94 (d, $J = 7.2$ Hz, 1H, H^{8}q.), 7.84–7.76 (m, 2H, H^3, H^{5}ph.), 7.70 (d, $J = 8.0$ Hz, 1H, H^{6}ph.), 7.65 (t, $J = 7.6$ Hz, 1H, H^{4}ph.), 7.58 (t, $J = 7.5$ Hz, 1H, H^{6}q.). LC-MS: $m/z = 273$ $[M + H]^+$. Anal. Calcd. for $C_{14}H_9ClN_2S$ C, 61.65; H, 3.33; N, 10.27; S, 11.76. Found: C, 61.67; H, 3.31; N, 10.26; S, 11.73.

2-(3-Chlorophenyl)-3H-quinazoline-4-thione (**2.14**). Yellow solid. Yield: 62.5%, m.p. 223–224 °C. IR (cm^{-1}): 3141, 3107, 3078, 3033, 2979, 2920, 1985, 1955, 1923, 1889, 1834, 1770, 1723, 1681, 1615, 1589, 1567, 1504, 1475, 1438, 1423, 1348, 1299, 1253, 1221, 1155, 1136, 1097, 1082, 1028, 998, 958, 908, 890, 883, 861, 800, 785, 765, 717, 696, 678, 650. ^{1}H NMR: δ (ppm) 8.74 (d, $J = 8.2$ Hz, 1H, H^{5}q.), 8.10 (t, $J = 8.3$ Hz, 1H, H^{7}q.), 7.94 (d, $J = 7.9$ Hz, 1H, H^{8}q.), 7.84–7.76 (m, 2H, H^2, H^{5}ph.), 7.70 (d, $J = 8.0$ Hz, 1H, H^{6}ph.), 7.65 (t, $J = 6.9$ Hz, 1H, H^{4}ph.), 7.58 (t, $J = 7.5$ Hz, 1H, H^{6}q.). LC-MS: $m/z = 373$ $[M + H]^+$. Anal. Calcd. for $C_{14}H_9ClN_2S$ C, 61.65; H, 3.33; N, 10.27; S, 11.76. Found: C, 61.64; H, 3.32; N, 10.25; S, 11.74.

2-(4-Chlorophenyl)-3H-quinazoline-4-thione (**2.15**). Yellow solid. Yield: 17.8%, m.p. 239–247 °C. IR (cm^{-1}): 3139, 3110, 3079, 3048, 3029, 2980, 2913, 2850, 2497, 1963, 1935, 1909, 1709, 1596, 1571, 1563, 1504, 1488, 1464, 1433, 1400, 1345, 1316, 1298, 1276, 1251, 1221, 1181, 1152, 1131, 1115, 1090, 1075, 1027, 1011, 955, 888, 861, 828, 798, 770, 759, 747, 726, 694, 685, 665, 629. ^{1}H NMR: δ (ppm) 10.24 (br s, 1H, NH), 8.73 (d, $J = 8.1$ Hz, 1H, H^{5}q.), 8.08 (t, 1H, $J = 7.8$ Hz, H^{7}q.), 7.93 (d, $J = 8.0$ Hz, 1H, H^{8}q.), 7.78 (d, $J = 8.0$ Hz, 2H, H^2, H^{6}ph.), 7.72–7.65 (m, 2H, H^3, H^{5}ph.), 7.60 (t, $J = 7.5$ Hz, 1H, H^{6}q.). LC-MS: $m/z = 273$ $[M + H]^+$. Anal. Calcd. for $C_{14}H_9ClN_2S$ C, 61.65; H, 3.33; N, 10.27; S, 11.76. Found: C, 61.69; H, 3.35; N, 10.22; S, 11.76.

2-(3-Fluorophenyl)-3H-quinazoline-4-thione (**2.16**). Yellow solid. Yield: 31.03%, m.p. 143–145 °C. IR (cm^{-1}): 3082, 3061, 3012, 2954, 2918, 2850, 2497, 1940, 1868, 1799, 1746, 1682, 1655, 1601, 1589, 1563, 1533, 1482, 1456, 1441, 1325, 1312, 1297, 1266, 1238, 1214, 1169, 1159, 1087, 1040, 1021, 998, 972, 957, 918, 885, 866, 796, 781, 759, 679, 640. ^{1}H NMR: δ (ppm) 13.95 (br s, 1H, NH), 8.68 (d, $J = 8.1$ Hz, 1H, H^{5}q.), 8.17 (m, 2H, H^2, H^{5}ph.), 8.06 (t, $J = 7.1$ Hz, 1H, H^{7}q.), 7.94 (d, $J = 7.4$ Hz, 1H, H^{8}q.), 7.73 (d, $J = 7.2$ Hz, 1H, H^{6}q.), 7.46 (dd, $J_1 = 17.7$, $J_2 = 9.0$ Hz, H^4, H^{6}ph.). LC-MS: $m/z = 256$ $[M + H]^+$. Anal. Calcd. for $C_{14}H_9FN_2S$ C, 65.61; H, 3.54; N, 10.93; S, 12.51. Found: C, 65.65; H, 3.57; N, 11.01; S, 12.46.

2-(4-Trifluoromethylphenyl)-3H-quinazoline-4-thione (**2.17**). Red solid. Yield: 46.7%, m.p. 122–123 °C. IR (cm^{-1}): 3043, 3029, 3000, 2917, 2848, 2516, 1987, 1947, 1928, 1907, 1870, 1839, 1651, 1613, 1599, 1558, 1534, 1448, 1406, 1319, 1308, 1247, 1205, 1159, 1103, 1064, 1009, 936, 909, 839, 826, 769, 676, 644, 629, 615. ^{1}H NMR: δ (ppm) 13.74 (br s, 1H, NH), 8.73 (d, $J = 8.1$ Hz, 1H, H^{5}q.), 8.30 (d, $J = 8.1$ Hz, 2H, H^2, H^{6}ph.), 8.00 (t, 1H, $J = 7.5$ Hz, H^{7}q.), 7.93 (d, $J = 8.0$ Hz, 1H, H^{8}q.), 7.89 (d, $J = 8.2$ Hz, 2H, H^3, H^{5}ph.), 7.68 (t, $J = 7.5$ Hz, 1H, H^{6}q.). LC-MS: $m/z = 306$ $[M + H]^+$. Anal. Calcd. for $C_{15}H_9F_3N_2S$ C, 58.82; H, 2.96; N, 9.15; S, 10.47. Found: C, 58.84; H, 3.01; N, 9.13; S, 10.44.

2-(3,4-Dimethoxyphenyl)-3H-quinazoline-4-thione (**2.18**). Yellow solid. Yield: 75.5%, m.p. 216–217 °C. IR (cm^{-1}): 3212, 3119, 3065, 2999, 2974, 2929, 2840, 2501, 1741, 1597, 1566, 1513, 1497, 1470, 1451, 1434, 1415, 1356, 1283, 1259, 1244, 1226, 1207, 1174, 151, 1076, 1022, 965, 935, 872, 861, 818, 770, 756, 711, 634, 616. ^{1}H NMR: δ (ppm) 13.76 (br s, 1H, NH), 8.61 (d, $J = 7.9$ Hz, 1H, H^{5}q.), 7.93–7.83 (m, 3H, H^2, H^{6}ph., H^{7}q.), 7.76 (d, $J = 8.0$ Hz, 1H, H^{8}q.), 7.57 (t, $J = 7.5$ Hz, 1H, H^{6}q.), 7.14 (d, $J = 8.5$ Hz, 1H, H^{5}ph.), 3.92 (s, 3H, 3-OCH$_3$), 3.87 (s, 3H, 4-OCH$_3$). LC-MS: $m/z = 299$ $[M + H]^+$. Anal. Calcd. for $C_{16}H_{14}N_2O_2S$ C, 64.41; H, 4.73; N, 9.39; S, 10.75. Found: C, 64.43; H, 4.75; N. 9.37; S, 10.72.

*Preparation of 2-R-(quinazolin-4-ylthio)carboxylic acids (**3.1–3.8**).* An appropriate amount of 2-R-quinazolin-4(3*H*)-thione (5 mmol) was added to methanol or ethanol (15 ml) with metallic potassium (0.39 g, 10 mmol). After the dissolution, the appropriate halogenocarboxylic acid (5 mmol) was added. The resulting mixture was refluxed for 1–5 h. After cooling to room temperature the hydrochloric acid was added to reach pH 4–5. The crystalline precipitate was filtered and reprecipitated.

*(Quinazolin-4-ylthio)acetic acid (**3.1**).* Orange solid. Yield: 63.6%, m.p. 192–194 °C. IR (cm^{-1}): 3115, 3059, 2982, 2930, 2786, 2427, 2319, 1865, 1697, 1661, 1610, 1571, 1537, 1489, 1469, 1406, 1373, 1320, 1300, 1263, 1235, 1199, 1165, 1114, 1021, 1001, 970, 921, 907, 895, 880, 858, 815, 766, 681, 654. ^{1}H NMR: δ (ppm) 12.90 (br s, 1H, COOH), 9.04 (s, 1H, H^{2}q.), 8.13 (d, $J = 7.9$ Hz, 1H, H^{5}q.), 8.08–7.98 (m, 2H, H^7, H^{8}q.), 7.79 (t, $J = 7.5$ Hz, 1H, H^{6}q.), 4.30 (s, 2H, SCH_2). LC-MS: *m/z* = 221 $[M+H]^+$. Anal. Calcd. for $C_{10}H_8N_2O_2S$ C, 54.53; H, 3.66; N, 12.72; S, 14.56. Found: C, 54.52; H, 3.64; N, 12.75; S, 14.57.

*2-Methyl-(quinazolin-4-ylthio)acetic acid (**3.2**).* Orange solid. Yield: 62.8%, m.p. 178–180 °C. IR (cm^{-1}): 3167, 3110, 3030, 2978, 2917, 2850, 2395, 2312, 1909, 1715, 1673, 1611, 1572, 1553, 1500, 1486, 1466, 1393, 1339, 1291, 1252, 1219, 1173, 1158, 1029, 998, 930, 901, 859, 770, 752, 689, 665, 645, 604. ^{1}H NMR: δ (ppm) 12.87 (br s, 1H, COOH), 8.08 (d, $J = 7.9$ Hz, 1H, H^{5}q.), 7.89 (d, $J = 7.6$ Hz, 1H, H^{8}q.), 7.97 (t, $J = 7.6$ Hz, 1H, H^{7}q.), 7.68 (t, $J = 7.4$ Hz, 1H, H^{6}q.), 4.19 (s, 2H, SCH_2), 2.68 (s, 3H, CH_3). LC-MS: *m/z* = 235 $[M+H]^+$. Anal. Calcd. for $C_{11}H_{10}N_2O_2S$ C, 56.40; H, 4.30; N, 11.96; S, 13.69. Found: C, 56.39; H, 4.31; N, 12.00; S, 13.64.

*2-Ethyl-(quinazolin-4-ylthio)acetic acid (**3.3**).* Yellow solid. Yield: 37.7%, m.p. 175–180 °C. IR (cm^{-1}): 2971, 2928, 2422, 2309, 1916, 1711, 1612, 1567, 1547, 1486, 1468, 1350, 1298, 1260, 1217, 1187, 1164, 1152, 1057, 1028, 988, 904, 889, 856, 763, 706, 668, 642, 624. ^{1}H NMR: δ (ppm) 14.72 (br s, 1H, COOH), 8.07 (d, $J = 8.2$ Hz, 1H, H^{5}q.), 7.98–7.91 (m, 1H, H^{7}q.), 7.88 (d, $J = 8.2$ Hz, 1H, H^{8}q.), 7.66 (t, $J = 7.5$ Hz, 1H, H^{6}q.), 4.17 (s, 2H, SCH_2), 2.93 (q, $J = 7.5$ Hz, 2H, CH_2CH_3), 1.38–1.28 (m, 3H, CH_2CH_3). LC-MS: *m/z* = 249 $[M+H]^+$. Anal. Calcd. for $C_{12}H_{12}N_2O_2S$ C, 58.05; H, 4.87; N, 11.28; S, 12.91. Found: C, 12.91; H, 4.82; N, 11.31; S, 12.93.

*2-Phenyl-(quinazolin-4-ylthio)acetic acid (**3.4**).* Yellow solid. Yield: 59.0%, m.p. 168–170 °C. IR (cm^{-1}): 3117, 3053, 3027, 2953, 2917, 2852, 1667, 1601, 1565, 1540, 1505, 1483, 1469, 1450, 1435, 1345, 1298, 1285, 1253, 1242, 1222, 189, 1147, 1104, 1081, 1030, 944, 926, 889, 858, 812, 760, 699, 687, 659, 619. ^{1}H NMR: δ (ppm) 12.95 (br s, 1H, COOH), 8.55 (d, $J = 8.04$ Hz, 2H, H^2, H^{6}ph.), 8.13 (d, $J = 8.15$ Hz, 1H, H^{5}q.), 8.03–7.94 (m, 2H, H^7, H^{8}q.), 7.71 (t, $J = 7.56$ Hz, 1H, H^{6}q.), 7.58–7.51 (m, 3H, H$^{3-5}$ph.), 4.28 (s, 2H, SCH_2). LC-MS: *m/z* = 297 $[M+H]^+$. Anal. Calcd. for $C_{15}H_{11}N_2O_2S$ C, 63.59; H, 3.91; N, 9.89; S, 11.32. Found: C, 63.57; H, 3.94; N, 9.89; S, 11.30.

*2-Benzyl-(quinazolin-4-ylthio)acetic acid (**3.5**).* White solid. Yield: 62.1%, m.p. 175–178 °C. IR (cm^{-1}): 3140, 3089, 3060, 3031, 2972, 2914, 2893, 2871, 1672, 1652, 1619, 1599, 1572, 1508, 1495, 1465, 1453, 1415, 1338, 1319, 1254, 1233, 1183, 1156, 1123, 1073, 1029, 999, 959, 920, 887, 867, 842, 828, 765, 731, 715, 696, 669, 657, 644, 611. LC-MS: *m/z* = 311 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{14}N_2O_2S$ C, 65.79; H, 4.55; N, 9.03; S, 10.33. Found: C, 66.01; H, 4.53; N, 9.00; S, 10.35.

*2-Phenethyl-(quinazolin-4-ylthio)acetic acid (**3.6**).* Pink solid. Yield: 15.0%, m.p. 171–173 °C. IR (cm^{-1}): 3028, 2989, 2927, 2858, 2761, 2657, 2499, 1942, 1713, 1614, 1569, 1553, 1488, 1458, 1382, 1358, 1295, 1188, 1162, 1077, 1030, 1011, 998, 937, 907, 887, 861, 788, 775, 761, 740, 698, 655, 625. ^{1}H NMR: δ (ppm) 12.97–12.77 (br s, 1H, COOH), 8.09 (d, $J = 8.0$ Hz, 1H, H^{5}q.), 7.95 (t, $J = 7.1$ Hz, 1H, H^{7}q.), 7.89 (d, $J = 8.2$ Hz, 1H, H^{8}q.), 7.68 (t, $J = 6.8$ Hz, 1H, H^{6}q.), 7.30–7.19 (m, 4H, H^2, H^3, H^5, H^{6}ph.), 7.17 (t, $J = 6.0$ Hz, 1H, H^{4}ph.), 4.18 (s, 2H, SCH_2), 3.20 (m, 4H, CH_2CH_2). LC-MS: *m/z* = 325 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{16}N_2O_2S$ C, 66.65; H, 4.97; N, 8.64; S, 9.88. Found: C, 66.64; H, 5.00; N, 8.64; S, 9.87.

*2-Styryl-(quinazolin-4-ylthio)acetic acid (**3.7**).* Red solid. Yield: 63.9%, m.p. 208–210 °C. IR (cm^{-1}): 3062, 3027, 2929, 1714, 1671, 1635, 1609, 1580, 1563, 1536, 1485, 1454, 1382, 1347, 1298, 1263, 1204, 1146, 1105, 1072, 1027, 997, 974, 944, 908, 869, 846, 753, 697, 679, 649, 621, 611. ^{1}H NMR: δ (ppm) 12.99 (br s, 1H, COOH), 8.16 (d, $J = 7.6$ Hz, 1H, HetCH=), 8.08 (d, $J = 8.0$ Hz, 1H, H^{5}q.), 7.98–7.87 (m, 2H, H^7, H^{8}q.), 7.80–7.71 (m, 3H, H^2, H^{6}ph., H^{6}q.), 7.53–7.42 (m, 3H, H$^{3-5}$ph.), 7.33 (d, $J = 7.6$ Hz, 1H, $=CHC_6H_5$), 4.23 (s, 2H, SCH_2). LC-MS: *m/z* = 323 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{14}N_2O_2S$ C, 67.06; H, 4.38; N, 8.69; S, 9.95. Found: C, 67.05; H, 4.38; N, 8.72; S, 9.99.

*2-Trifluoromethyl-(quinazolin-4-ylthio)acetic acid (**3.8**).* White solid. Yield: 92.6%, m.p. 240–242 °C. IR (cm^{-1}): 2997, 2920, 2712, 2595, 1712, 1612, 1566, 1488, 1416, 1394, 1381, 1352, 1303, 1264, 1248, 1223, 1187, 1149, 1112, 1026, 1003, 963, 915, 883, 850, 801, 769, 756, 734, 693, 673, 629, 610. ^{1}H NMR: δ (ppm) 12.97 (br s, 1H, COOH), 8.25 (d, $J = 8.2$ Hz, 1H, H^{5}q.), 8.13–8.04 (m, 2H, H^7, H^{8}q.), 7.90 (t, $J = 8.1$ Hz, 1H, H^{6}q.), 4.23 (s, 2H, SCH_2). LC-MS: *m/z* = 289 $[M+H]^+$. Anal. Calcd. for $C_{11}H_7F_3N_2O_2S$ C, 45.84; H, 2.45; N, 9.72; S, 11.12. Found: C, 45.85; H, 2.43; N, 9.72; S, 11.13.

*2-Morpholin-(quinazolin-4-ylthio)acetic acid (**3.9**).* Yellow solid. Yield: 29.5%, m.p. 120–121 °C. IR (cm^{-1}): 2965, 2926, 2861, 2836, 1624, 1582, 1559, 1513, 1488, 1472, 1440, 1410, 1374, 1360, 1338, 1303, 1260, 1222, 1162, 1151, 1113, 1101, 1070, 1020, 996, 931, 915, 862, 834, 794, 769, 736, 682, 666, 642. ^{1}H NMR: δ (ppm) 12.95 (br s, 1H, COOH), 8.06 (d, $J = 8.2$ Hz, 1H, H^{5}q.), 8.00 (d, $J = 7.2$ Hz, 1H, H^{8}q.), 7.87 (t, $J = 7.5$ Hz, 1H, H^{7}q.), 7.43 (t, $J = 8.0$ Hz, 1H, H^{6}q.), 4.23 (s, 2H, SCH_2), 4.05 (s, 4H, $N(CH_2)_2$), 3.71 (s, 4H, $O(CH_2)_2$). LC-MS: *m/z* = 306 $[M+H]^+$. Anal. Calcd. for $C_{14}H_{15}N_3O_3S$ C, 55.07; H, 4.95; N, 13.76; S, 10.50. Found: C, 55.02; H, 4.96; N, 13.78; S, 10.51.

*Preparation of 2-R-(quinazolin-4-ylthio)carboxylic acids amides (**4.1–4.9**).* An appropriate amount of 2-R-quinazolin-4(3*H*)-thione (5 mmol) was added to ethanol or methanol (20 ml) with metallic potassium (0.39 g, 10 mmol).

Then the appropriate amide of halogenocarboxylic acid (10 mmol) was added and refluxed for 1–2 h. After cooling to room temperature the mixture was poured into the cold water. The crystalline precipitate was filtered. Substances were recrystallized from a mixture of 2-propanol–water (1:3) or from 2-propanol.

*N-Phenyl-2-(quinazolin-4-ylthio)acetamide (**4.1**).* Yellow solid. Yield: 88.0%, m.p. 121–124 °C. IR (cm^{-1}): 3298, 3194, 3144, 3058, 2954, 2922, 2850, 1662, 1598, 1535, 1497, 1485, 1444, 1421, 1358, 1319, 1279, 1242, 1180, 1164, 1147, 1113, 1079, 1020, 996, 956, 904, 886, 869, 857, 836, 791, 757, 692, 677, 652, 616. ^{1}H NMR: δ (ppm) 10.49 (br s, 1H, NH), 8.99 (s, 1H, H^{2}q.),

8.19 (d, $J = 8.1$ Hz, 1H, H^5q.), 8.01 (t, $J = 7.4$ Hz, 2H, H^7, H^8q.), 7.77 (d, $J = 7.4$ Hz, 1H, H^6q.), 7.67–7.57 (m, 2H, H^2, H^4ph.), 7.31 (t, $J = 7.2$ Hz, 2H, H^3, H^5ph.), 7.25–7.19 (m, 1H, H^4ph.), 4.41 (s, 2H, SCH_2). LC-MS: $m/z = 296$ $[M+H]^+$. Anal. Calcd. for $C_{16}H_{13}N_3OS$ C, 65.06; H, 4.44; N, 14.23; S, 10.86. Found: C, 65.03; H, 4.45; N, 14.23; S, 10.81.

N-Phenyl-2-(quinazolin-4-ylthio)propionamide (**4.2**). Yellow solid. Yield: 80.8%, m.p. 138–140 °C. IR (cm^{-1}): 3237, 3191, 3131, 3061, 3043, 2979, 2928, 2871, 2854, 1658, 1602, 1560, 1545, 1485, 1445, 1376, 1334, 1320, 1294, 1280, 1254, 1229, 1188, 1163, 1089, 1075, 1029, 990, 958, 931, 900, 869, 835, 790, 758, 713, 696, 679, 652. ^{1}H NMR: δ (ppm) 10.47 (br s, 1H, NH), 9.05 (s, 1H, H^2q.), 8.14 (d, $J = 8.2$ Hz, 1H, H^5q.), 8.06–7.95 (m, 2H, H^7, H^8q.), 7.75 (t, $J = 7.4$ Hz, 1H, H^6q.), 7.63 (d, $J = 8.2$ Hz, 2H, H^2, H^6ph.), 7.33 (t, $J = 7.5$ Hz, 2H, H^3, H^5ph.), 7.08 (t, $J = 7.0$ Hz, 1H, H^4ph.), 5.06 (q, $J = 6.6$ Hz, 1H, SCH), 1.69 (d, $J = 6.9$ Hz, 3H, $SCHCH_3$). LC-MS: $m/z = 310$ $[M+H]^+$. Anal. Calcd. for $C_{17}H_{15}N_3OS$ C, 66.00; H, 4.89; N, 13.58; S, 10.36. Found: C, 66.01; H, 4.87; N, 13.57; S, 10.38.

N-Benzyl-2-(quinazolin-4-ylthio)propionamide (**4.3**). Yellow solid. Yield: 74.2%, m.p. 100–160 °C. IR (cm^{-1}): 3320, 3270, 3063, 3032, 2972, 2921, 2853, 1645, 1615, 1597, 1551, 1496, 1483, 1451, 1420, 1396, 1375, 1360, 1331, 1319, 1303, 1278, 1257, 1237, 1219, 1187, 1162, 1150, 1099, 1078, 1045, 1022, 995, 959, 909, 867, 835, 810, 790, 758, 732, 695, 679, 651, 618. ^{1}H NMR: δ (ppm) 8.98 (s, 1H, H^2q.), 8.80 (br s, 1H, NH), 8.12 (d, $J = 8.2$ Hz, 1H, H^5q.), 8.06–7.95 (m, 2H, H^7, H^8q.), 7.74 (t, $J = 7.8$ Hz, 1H, H^6q.), 7.38–7.20 (m, 5H, H^{2-6}ph.), 4.97–4.88 (m, 1H, SCH), 4.33 (d, $J = 5.8$ Hz, 2H, CH_2), 1.63 (d, $J = 7.0$ Hz, 3H, $SCHCH_3$). LC-MS: $m/z = 324$ $[M+H]^+$. Anal. Calcd. for $C_{18}H_{17}N_3OS$ C, 66.85; H, 5.30; N, 12.99; S, 9.91. Found: C, 66.85; H, 5.32; N, 12.96; S, 9.87.

2-(2-Methyl-quinazolin-4-ylthio)-N-phenylacetamide (**4.4**). Yellow solid. Yield: 64.6%, m.p. 178–180 °C. IR (cm^{-1}): 3271, 3189, 3130, 3055, 3030, 2977, 2907, 2849, 1652, 1597, 1548, 1531, 1495, 1479, 1443, 1396, 1382, 1365, 1330, 1318, 1292, 1244, 1208, 1162, 1148, 1075, 1027, 1015, 991, 972, 921, 906, 872, 842, 775, 752, 719, 687, 666, 638. ^{1}H NMR: δ (ppm) 10.43 (br s, 1H, NH), 8.11 (d, $J = 8.1$ Hz, 1H, H^5q.), 7.95 (t, $J = 7.6$ Hz, 1H, H^7q.), 7.86 (d, $J = 8.2$ Hz, 1H, H^8q.), 7.67 (t, $J = 7.5$ Hz, 1H, H^6q.), 7.61 (d, $J = 7.8$ Hz, 2H, H^2, H^6ph.), 7.33 (t, $J = 7.8$ Hz, 2H, H^3, H^5ph.), 7.07 (t, $J = 7.3$ Hz, 1H, H^4ph.), 4.32 (s, 2H, SCH_2), 2.67 (s, 3H, CH_3). LC-MS: $m/z = 310$ $[M+H]^+$. Anal. Calcd. for $C_{17}H_{15}N_3OS$ C, 66.00; H, 4.89; N, 13.58; S, 10.36. Found: C, 66.03; H, 4.91; N, 13.64; S, 10.32.

3-(2-Methyl-quinazolin-4-ylthio)-N-phenylpropionamide (**4.5**). Yellow solid. Yield: 46.4%, m.p. 199–201 °C. IR (cm^{-1}): 3246, 3186, 3121, 3060, 3023, 2971, 2917, 2849, 1682, 1605, 1546, 1480, 1443, 1370, 1336, 1306, 1282, 1253, 1209, 1175, 1146, 1080, 1029, 993, 965, 921, 906, 866, 846, 787, 753, 695, 667, 640. ^{1}H NMR: δ (ppm) 10.00 (br s, 1H, NH), 8.04 (d, $J = 8.2$ Hz, 1H, H^5q.), 7.96–7.89 (m, 1H, H^7q.), 7.86 (d, $J = 8.3$ Hz, 1H, H^8q.), 7.68–7.57 (m, 3H, H^2, H^6ph., H^6q.), 7.30 (t, $J = 7.3$ Hz, 2H, H^3, H^5ph.), 7.04 (t, $J = 7.0$ Hz, 1H, H^4ph.), 3.63 (t, $J = 6.2$ Hz, 2H, SCH_2), 2.89 (t, $J = 6.2$ Hz, 2H, SCH_2CH_2), 2.70 (s, 3H, CH_3). LC-MS: $m/z = 324$ $[M+H]^+$. Anal. Calcd. for $C_{18}H_{17}N_3OS$ C, 66.85; H, 5.30; N, 12.99; S, 9.91. Found: C, 66.84; H, 5.31; N, 13.02; S, 9.94.

2-(2-Methyl-quinazolin-4-ylthio)-N-phenylpropionamide (**4.6**). Yellow solid. Yield: 72.4%, m.p. 119–130 °C. IR (cm^{-1}): 3455, 3260, 3199, 3139, 3083, 3030, 2978, 2928, 2869, 1669, 1623, 1600, 1550, 1503, 1482, 1444, 1375, 1339, 1325, 1294, 1251, 1229, 1210, 1186, 1155, 1076, 1057, 1029, 991, 919, 869, 844, 799, 755, 716, 694, 669, 641. ^{1}H NMR: δ (ppm) 10.41 (br s, 1H, NH), 8.06 (d, $J = 8.1$ Hz, 1H, H^5q.), 7.95 (t, $J = 7.9$ Hz, 1H, H^7q.), 7.86 (d, $J = 8.2$ Hz, 1H, H^8q.), 7.72–7.60 (m, 3H, H^6q., H^2, H^6ph.), 7.33 (t, $J = 7.8$ Hz, 2H, H^3, H^5ph.), 7.08 (t, $J = 7.3$ Hz, 1H, H^4ph.), 4.93 (q, $J = 6.9$ Hz, 1H, SCH), 2.68 (s, 3H, CH_3), 1.68 (d, $J = 6.0$ Hz, 3H, $SCHCH_3$). LC-MS: $m/z = 368$ $[M+H]^+$. Anal. Calcd. for $C_{18}H_{17}N_3OS$ C, 66.85; H, 5.30; N, 12.99; S, 9.91. Found: C, 66.83; H, 5.32; N, 13.00; S, 9.91.

2-(2-Morpholin-4-ylmethyl-quinazolin-4-ylthio)-N-phenylacetamide (**4.7**). Yellow solid. Yield: 73.5%, m.p. 184–185 °C. IR (cm^{-1}): 3306, 3053, 2964, 2952, 2941, 2921, 2883, 2840, 2826, 1676, 1597, 1561, 1547, 1525, 1497, 1481, 1452, 1439, 1419, 1366, 1353, 1325, 1308, 1284, 1258, 1240, 1189, 1170, 1135, 1112, 1079, 1069, 1030, 1005, 984, 969, 957, 922, 896, 887, 874, 858, 837, 821, 774, 758, 704, 691, 650, 637, 605. ^{1}H NMR: δ (ppm) 10.38 (br s, 1H, NH), 8.15 (d, $J = 8.1$ Hz, 1H, H^5q.), 8.04–7.92 (m, 2H, H^7, H^8q.), 7.76 (t, $J = 7.8$ Hz, 1H, H^6q.), 7.61 (d, $J = 7.6$ Hz, 2H, H^2, H^6ph.), 7.32 (d, $J = 6.0$ Hz, 2H, H^3, H^5ph.), 7.06 (t, $J = 6.3$ Hz, 1H, H^4ph.), 4.32 (s, 2H, SCH_2), 3.75 (s, 2H, CH_2N), 3.40 (s, 4H, $N(CH_2)_2$), 2.55 (s, 4H, $O(CH_2)_2$). LC-MS: $m/z = 395$ $[M+H]^+$. Anal. Calcd. for $C_{21}H_{22}N_4O_2S$ C, 63.94; H, 5.62; N, 14.20; S, 8.13. Found: C, 63.94; H, 5.60; N, 14.27; S, 8.11.

N-(2-Chlorophenyl)-2-(2-methyl-quinazolin-4-ylthio)acetamide (**4.8**). Yellow solid. Yield: 85.5%, m.p. 188–190 °C. IR (cm^{-1}): 3329, 2895, 1680, 1611, 1585, 1552, 1530, 1479, 1470, 1456, 1436, 1417, 1380, 1318, 294, 1281, 1255, 1241, 1197, 1149, 1055, 1032, 989, 960, 935, 917, 898, 871, 857, 839, 789, 765, 753, 735, 680, 667, 640. ^{1}H NMR: δ (ppm) 9.89 (br s, 1H, NH), 8.12 (d, $J = 8.1$ Hz, 1H, H^5q.), 7.97 (t, $J = 7.3$ Hz, 1H, H^7q.), 7.88 (d, $J = 8.3$ Hz, 1H, H^8q.), 7.76 (d, $J = 7.8$ Hz, 1H, H^6ph.), 7.68 (t, $J = 7.5$ Hz, 1H, H^6q.), 7.50 (d, $J = 7.9$ Hz, 1H, H^3ph.), 7.34 (t, $J = 7.6$ Hz, 1H, H^4ph.), 7.20 (t, $J = 7.3$ Hz, 1H, H^5ph.), 4.39 (s, 2H, SCH_2), 2.70 (s, 3H, CH_3). LC-MS: $m/z = 344$ $[M+H]^+$. Anal. Calcd. for $C_{17}H_{14}ClN_3OS$ C, 59.39; H, 4.10; N, 12.22; S, 9.33. Found: C, 59.38; H, 4.13; N, 12.21; S, 9.36.

N-(4-Chlorophenyl)-2-(2-methyl-quinazolin-4-ylthio)acetamide (**4.9**). Yellow solid. Yield: 52.5%, m.p. 162–164 °C. IR (cm^{-1}): 3264, 3186, 3119, 3054, 2920, 2889, 2850, 1660, 1612, 1594, 1549, 1521, 1491, 1481, 1457, 1400, 1379, 1330, 1293, 1274, 1245, 1207, 1168, 1151, 1094, 1076, 1014, 991, 970, 920, 892, 841, 823, 765, 755, 734, 708, 697, 667, 639. ^{1}H NMR: δ (ppm) 10.57 (br s, 1H, NH), 8.11 (d, $J = 8.1$ Hz, 1H, H^5q.), 7.96 (t, $J = 7.6$ Hz, 1H, H^7q.), 7.87 (d, $J = 8.2$ Hz, 1H, H^8q.), 7.71–7.60 (m, 3H, H^6q., H^2, H^6ph.), 7.39 (t, $J = 6.8$ Hz, 2H, H^3, H^5ph.), 4.31 (s, 2H, SCH_2), 2.62 (s, 3H, CH_3). LC-MS: $m/z = 344$ $[M+H]^+$. Anal. Calcd. for $C_{17}H_{14}ClN_3OS$ C, 59.39; H, 4.10; N, 12.22; S, 9.33. Found: C, 58.41; H, 4.11; N, 12.25; S, 9.33.

Docking, scoring and visual inspection of synthesized substances into the enzymes binding site

Flexible molecular docking was carried out using the software package OpenEye, including related utilities: Fred Receptor2.2.5, Vida4.1.1, Flipper, Babel3, Omega2.4.3 and Fred2.2.5[19,20]. The crystal structures of the enzymes were obtained from the protein data bank[21].

The methodology of research consisted of the following steps:

– generation of R-, S- and *cis*-, *trans*-isomers of ligands (the studied compounds and relevant drugs, program Flipper),

which allowed the production of studied compounds' isomer's range;
- molecular modeling (Hyper Chem 7.5) by generation of the obtained isomeric forms' 3D-structures – using the method of molecular mechanics (MM +) and semi-empirical quantum mechanical method with Polak–Ribiere algorithm (PM3);
- generation of ligands conformations (Omega2.4.3). The number of obtained conformations was not significant due to the further selection of the most optimal conformers using the program Fred2.2.5;
- carrying out molecular docking (Fred2.2.5).

Scoring functions (Shapegauss, PLP, Chemgauss2, Chemgauss3, Chemscore, OEChemscore, Screenscore, CGO, CGT, Zapbind, Consensus Score) were obtained as a result of studies, the values of which assess specific characteristics of the ligand–protein complex, indicating the possibility of their matching.

Antimicrobial and antifungal activity *in vitro* testing

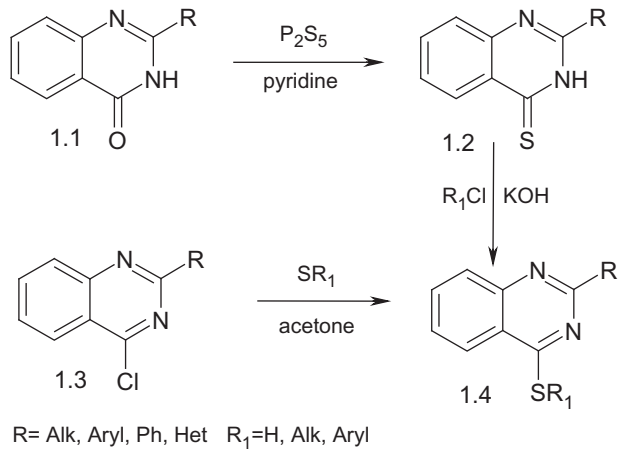
Materials and methods

The *in vitro* antibacterial activity of all newly synthesized compounds were tested in the Zaporozhye Regional Hospital Bacterial Laboratory against Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram negative bacteria (*Enterobacter aerogenes* 12*, Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* 68) and antifungal properties against *Candida albicans* ATCC 885653. The amount of microbial cells was 1.5×10^8 c.f.u./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 determination of the preliminary antibacterial and antifungal activity. Standard sterilized filter paper discs (6 mm diameter) were impregnated with a solution of the test compound in DMSO (100 μg/disk) and placed it on an agar (Müller–Hinton Broth (Oxoid)) plate seeded with the appropriate test organism in triplicates. DMSO alone was used as a control at the same above-mentioned concentration. Gatifloxacin, Gemifloxacin, Moxifloxacin, Ceftriaxone and Nystatin were used as reference drugs. The results were recorded for each of the tested compound as the average diameter of inhibition zones diameters (IZD) of bacterial or fungal growth around the discs in mm.

Anticancer activity testing

Materials and methods

Primary anticancer assay was performed against human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda[22]. 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 40 000 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 additions (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.



Scheme 1. Synthesis of 2-R-quinazolin-4(3*H*)-thiones and their derivatives.

Results and discussion

Chemistry

Among known methods of 2-R-quinazolin-4(3*H*)-thiones (**1.2**) synthesis are thionation of 2-R-quinazolin-4(3*H*)-ones (**1.1**) by phosphorus pentasulfide in acetone in the presence of potassium acetate or the alkylation of 4-chloroquinazoline (**1.3**) with appropriate thions (Scheme 1)[9].

Alkylation of 2-R-quinazolin-4(3*H*)-thiones (**1.2**) by the halogenoalkanes in an alkaline medium with the presence of tetrabutylammonium bromide leads to the corresponding S-derivatives (**1.4**) (Scheme 1)[23].

When isothiocyanates were used as electrophiles to react with *ortho*-bromophenyl isocyanides (**1.5**), appropriate cyclic 2-R-quinazolin-4(3*H*)-thiones (**1.7**) were formed in high yields (Scheme 2)[24]. The reaction mixture with isothiocyanates was gradually warmed to 40 °C before quenching with water to form intermediate **1.6**.

Thus, the variety of the known 2-R-quinazolin-4(3*H*)-thiones is quite narrow, so synthetic and biological investigation in this field is still novel and promising.

Synthesis of novel 2-R-quinazolin-4(3H)-thiones

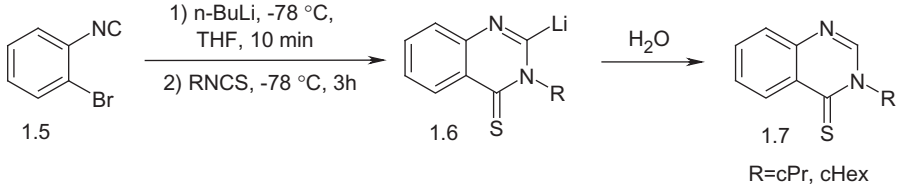
Thionation of 2-R-4(3*H*)-quinazolones was carried out with the help of a known reagent, namely Lawesson's (2,4-*bis*(*p*-methoxyphenyl)-1,3-dithiadiphosphetane-2,4-disulfide)[25,26], which reduced the reaction time and increased the yield of the required 2-R-quinazolin-4(3*H*)-thiones (**2.1–2.18**) (Scheme 3).

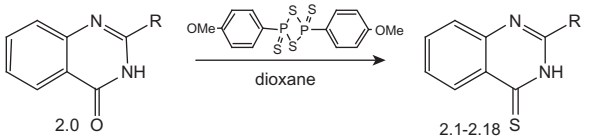
The structure of all synthesized compounds was evaluated by elemental analysis and their spectral data (IR, LC-MS, ^{1}H-NMR spectra).

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

In the IR-spectra of compounds **2.1–2.18**, the aromatic rings' stretchings were presented as the peaks of the weak and medium intensity absorption at 1620–1428 cm^{-1}. Furthermore, the existence of aromatic system was confirmed by C–H stretchings at 3025–3148 cm^{-1}. Out-of-plane vibrations of =C–H bond were observed in the range from 871 to 690 cm^{-1}. Methylene groups of **2.3**, **2.5**, **2.6**, **2.9**, **2.10**, **2.12** were characterized by deformational vibrations at 1465–1456 cm^{-1}, and methyl groups of **2.2**, **2.3**, **2.18** at 1378–1356 cm^{-1}. Methoxy group of compound **2.18** resonated as two intense stretchings: asymmetric at 1258–1243 cm^{-1} and symmetric at 1021 cm^{-1}. Signals of aliphatic C–N bond (**2.9**, **2.10**) were presented in the range from 1369 to 1000 cm^{-1},

Scheme 2. Synthesis of 3-R-quinazolin-4(3H)-thiones.





2.1 H	2.7 $CH{=}CH\text{-}C_6H_5$	2.13 $2\text{-}Cl\text{-}C_6H_4$
2.2 CH_3	2.8 CF_3	2.14 $3\text{-}Cl\text{-}C_6H_4$
2.3 C_2H_5	2.9 morpholin-4-yl	2.15 $4\text{-}Cl\text{-}C_6H_4$
2.4 C_6H_5	2.10 CH_2-morpholin-4-yl	2.16 $3\text{-}F\text{-}C_6H_4$
2.5 $CH_2\text{-}C_6H_5$	2.11 thiophen-3-yl	2.17 $4\text{-}CF_3\text{-}C_6H_4$
2.6 $(CH_2)_2\text{-}C_6H_5$	2.12 1H-imidazol-1-yl	2.18 $3,4\text{-}(OCH_3)\text{-}C_6H_3$

Scheme 3. Synthesis of 2-R-quinazolin-4(3H)-thiones.

whereas the C=N bond (**2.1–2.18**) absorbed approximately at the same frequencies as the C=C bond at 1685 to 1613 cm^{-1}, but broadened and more intensive. The vibration of chlorine substituent of compounds **2.13–2.15** was observed between 1097 and 1027 cm^{-1}, and the vibrations of fluorine substituents (**2.16**) were observed at 1266 to 1087 cm^{-1}. Multiplet of strong peaks in the range from 1360 to 1103 cm^{-1} was typical for CF_3-group for compounds **2.8** and **2.17**. The broadened and very weak absorption at 2571–2489 cm^{-1} only partially confirmed the presence of SH-groups of compounds, as vibrations overlapped with signals of other groups. The stretching of C=S bond of compound **2.11** was observed at 1250–1015 cm^{-1}.

^{1}H NMR-spectra data characterized substances **2.1–2.18** according to their proposed structures, and quinazoline's signals were found in the next range of ppm: one-proton doublets of H^5-quin. and H^8-quin. at the 8.74–8.44 and at the 7.94–7.44 respectively; H^7-quin. and H^6-quin. as one-proton triplets at the 8.10–7.67 and at the 7.76–7.26, and for unsubstituted quinazolin-4-(3*H*)-thione (**2.1**) H^2-quin. was observed at 8.19 ppm. The one-proton broadened singlet of the NH-group was detected at the 13.98–13.72 ppm, and for compounds **2.8, 2.9 2.13** it was shifted to stronger field – the 12.44 ppm, the 12.88 ppm and the 10.04 ppm, respectively. Signals of phenyl protons were presented at 8.30–7.14 ppm with proper multiplicity. The SC***H*** = CH signal of compound **2.7** was recorded at 7.29 ppm. Aliphatic morpholyl protons for **2.9** and **2.10** were observed in strong field at 3.76–2.53 ppm. The alkyl and methoxy groups were found at 3.92–1.27 ppm.

Synthesis of (2-R-quinazolin-4(3H)-ylthio)acetic acids

The (2-R-quinazolin-4(3*H*)-ylthio)acetic acids (**3.1–3.9**) were obtained by refluxing the appropriate 2-R-quinazolin-4(3*H*)-thiones (**2.1–2.9**) with chloroacetic acid in potassium alkoxide (Scheme 4).

The IR spectra of (2-R-quinazolin-4(3*H*)-ylthio)acetic acids (**3.1–3.9**) were characterized by high intensity bands of carbonyl group stretchings at 1715–1624 cm^{-1}, and for compounds **3.1, 3.2** and **3.5** the signal was observed as a doublet. The hydroxy group was characterized by a broad peak at 2964–2914 cm^{-1} (overlapping with CH vibrations). The weak intensity absorption band at 1635 cm^{-1} and medium at 1614–1416 cm^{-1} corresponds to double bonds and aromatic ring of compounds. Aromatic and vinyl CH group vibrations were observed at 3167–2965 cm^{-1}. Methylene groups of **3.1–3.9** had characteristic absorption peak at 1488–1458 cm^{-1}, and methyl groups of **3.2, 3.3** absorbed at 1392–1345 cm^{-1}. The stretching vibrations of =CH group appeared at 3062–3027 cm^{-1} and out-of-plane vibrations from 908 to 697 cm^{-1}. In compound **3.8,** the CF bond had a characteristic multiplet with strong bands in the range from 1352 to 1112 cm^{-1}. The C=N bond was characterized by broadened vibrations of variable intensity at 1667–1610 cm^{-1}.

In the ^{1}H NMR spectra, the substances **3.1–3.9** were characterized by broadened one-proton singlet of carboxylic group proton at 14.72–12.87 ppm. For **3.1,** the H^2-quin. signal was found at 9.04 ppm. The one-proton doublets of H^5-quin. and H^8-quin. appeared at 8.25–8.06 and 8.13–7.97 ppm; one-proton signals H^7-quin. and H^6-quin. were shown as triplets at 8.09–7.89 and 7.90–7.43 ppm, respectively. The signals of phenyl protons were also observed in the low field at 8.55–7.15 ppm. The *cis* CH=CH fragment of **3.7** was detected at 7.33 and 8.16 ppm, deshielded by substituents. Characteristic two-proton singlet of SCH_2-group was detected at 4.30–4.17 ppm. Aliphatic morpholyl protons of **3.9** were observed at 4.05–3.71 ppm as singlets, and protons of alkyl substituents of compounds **3.2–3.3**, **3.5–3.6**, **3.9** were observed in a strong field at 3.20–1.28 ppm with corresponding multiplicity.

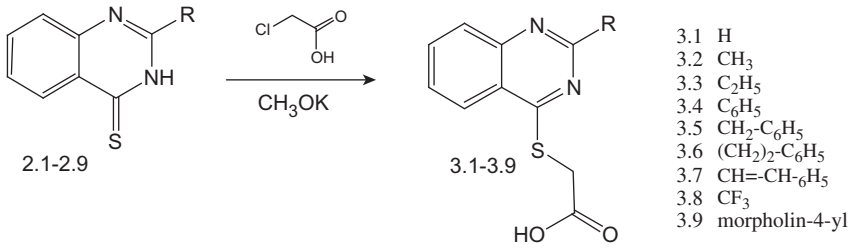
Synthesis of (2-R-quinazolin-4 (3H)-ylthio)carboxylic acids amides

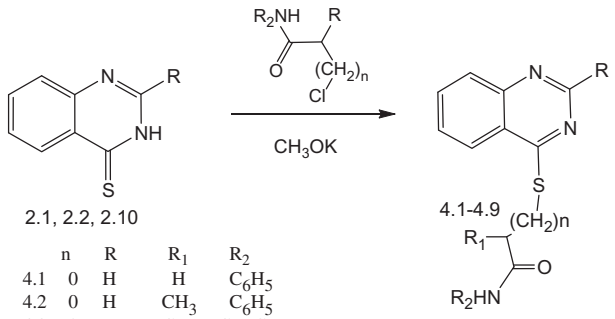
The next step of synthesis was refluxing the 2-R-quinazolin-4(3*H*)-thiones (**2.1, 2.2, 2.10**) with halogenocarboxylic acids amides in potassium methylate to obtain the appropriate (2-R-quinazolin-4(3*H*)-ylthio)carboxylic acids amides (**4.1–4.9**) (Scheme 5).

In the IR-spectra, the amide NH-proton of compounds **4.1–4.9** was characterized by broadened and medium intensive signal at 3245–3455 cm^{-1}. The carbonyl vibrations were observed at 1682–1645 cm^{-1}. Double bonds of aromatic rings showed medium strength absorption peaks at 1615–1439 cm^{-1}. Aromatic and vinyl CH-groups occurred at 3329–3023 cm^{-1}, and aliphatic CH for compounds **4.2, 4.3, 4.6** at 2979–2853 cm^{-1}. Methylene groups of compounds **4.1, 4.3–4.5, 4.7–4.9** had characteristic absorption peak at 1485–1479 cm^{-1}, and methyl groups of compounds **4.2–4.6, 4.8, 4.9** at 1380–1370 cm^{-1}. The signals of CN-bond appeared in the range from 1353 to 1005 cm^{-1}, and C=N bond at 1682–1611 cm^{-1}. Chlorine substituted phenyls **4.8** and **4.9** had a characteristic valence vibrations at 1094–1032 cm^{-1}.

In the ^{1}H NMR spectra of (2-R-quinazolin-4(3*H*)-ylthio)carboxylic acids amides (**4.1–4.9),** NH-proton was shown almost for all amides as broadened one-proton singlet at 10.57–9.89 ppm, except the compound **4.3** (8.80 ppm). The H^5 and H^8 quin. signals were found at 8.06–7.89 and 8.01–7.90 ppm, H^7 and H^6 at 8.01–7.90 and 7.77–7.62 ppm; H^2 quin. for **4.1–4.3** at 9.05–8.98 ppm. The SC*H*- of **4.2, 4.3, 4.6** resonated as one-proton quadruplet at 5.06–4.85 ppm. The SCH_2-group appeared as a triplet for **4.5** at 3.63 ppm and two-proton singlet at 4.41–4.31 ppm for all other

Scheme 4. Synthesis of (2-R-quinazolin-4(3H)-ylthio)acetic acids.





	n	R	R_1	R_2
4.1	0	H	H	C_6H_5
4.2	0	H	CH_3	C_6H_5
4.3	0	H	CH_3	$CH_2C_6H_5$
4.4	0	CH_3	H	C_6H_5
4.5	1	CH_3	H	C_6H_5
4.6	0	CH_3	CH_3	C_6H_5
4.7	0	CH_2-morpholin-4-yl	H	C_6H_5
4.8	0	CH_3	H	4-C1-C_6H_5
4.9	0	CH_3	H	2-C1-C_6H_5

Scheme 5. Synthesis of the (2-R-quinazolin-4(3H)-ylthio)carboxylic acids amides.

compounds. The signals of phenyl protons were presented at 7.61–7.04 ppm. Aliphatic signals were observed in the strong field (2.89–1.63 ppm).

Pharmacology

Docking studies to Candida albicans dihydrofolate reductase. In continuation of our potential antimicrobials investigation, the dihydrofolate reductase (DHFR) was used as a model to study compounds' affinity to it. DHFR is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as electron donor[27]. A variety of drugs act as its inhibitors: the antibiotic trimethoprim and its derivatives, the antimalarial drugs pyrimethamine and proguanil, the chemotherapeutic agent methotrexate[28]. Thus, we were aimed to perform the docking studies, as an approach to find the molecules with affinity to a specific biological target, namely, synthesized compounds into the active site of DHFR to predict the possible presence of the antimicrobial activity.

Investigation was conducted by 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[19,20]. The crystal structure of the enzyme DHFR (1AOE.pdb) was obtained from the protein data bank[21]. The 7-(pentan-3-yl)-7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine (GW345) was used as a reference (Figure 1)[27].

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

The analysis of docking interactions between DHFR and investigated compounds revealed the best results for 2-(2-methylquinazolin-4-ylthio)-*N*-phenylacetamide (**4.6**) (Figure 1). Also, the Consensus Score of 2-(2-morpholin-4-ylmethylquinazolin-4-ylthio)-*N*-phenylacetamide (**4.7**) was at the same level as the reference (Table 1).

By generalizing obtained results, amides had better affinity to DHFR. The more planar substituents the molecules had the better was the affinity. Introduction of simple alkyl radicals worsens it. Therefore, to prove the presence of antimicrobial activity, the next step of investigation was *in vitro* screening.

Antimicrobial and antifungal activity study. All the newly synthesized compounds were evaluated for *in vitro* antibacterial activity against Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram negative bacteria (*Enterobacter aerogenes* 12, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* 68) and antifungal properties against *Candida albicans* ATCC 885653 (Table 2).

As a result, it was found that *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* were insensitive to all the synthesized compounds. Moreover, compounds **2.5–2.7**, **2.12–2.14**, **2.16–2.18**, **3.1–3.9**, **4.1–4.9** had no antimicrobial and antifungal effects on all studied microorganisms.

Substances **2.2–2.4**, **2.9–2.11**, **2.15** had the most significant influence on the growth of *C. albicans*, showing the antifungal activity. 2-Methylquinazolin-4-(3*H*)-thione (**2.2**) possessed the best results of inhibition zone diameter, 23 mm, exceeding 21 mm of Nystatin (Table 1). The growth of *S. aureus* and *E. aeruginosa* were slightly suppressed by compounds **2.1**, **2.4**, **2.8** and **2.9**. Interestingly, the most active among them appeared to be unsubstituted quinazolin-4(3*H*)-thione (**2.1**), which delayed their growth at 15 and 11 mm, respectively. The latter one (**2.1**) and 2-phenylquinazolin-4(3*H*)-thione (**2.4**) also moderately inhibited the growth of *K. pneumonia* at 11 and 10 mm. But, unfortunately, they did not suppress the level of any of the references even at a concentration of 100 μg/disk.

Hence, the studies have shown that the most active antimicrobial and antifungal compounds were: unsubstituted (**2.1**), 2-methyl-(**2.2**) and 2-phenylquinazolin-4(3*H*)-thione (**2.4**).

Structure–antimicrobial activity correlation. Some regularity in the appearance of biological activity could be traced. The introduction of larger substituent in the second position of quinazolin-4(3*H*)-thione gives a significant weakening of the antibacterial action against *S. aureus*, *E. faecalis, K. pneumoniae* and results in a moderate antifungal action against *C. albicans*. The modification of forth position with carboxylic acids and amides residues surprisingly leads to the disappearance of any antimicrobial activity. Analyzing the molecular docking results, there was no correlation between affinity to DHFR and antimicrobial data. Thus, we could suppose that synthesized compounds had other mechanism of antimicrobial activity.

Docking studies of CK2 kinase. Most interesting target for anticancer investigations is protein kinase CK2 (casein kinase II),

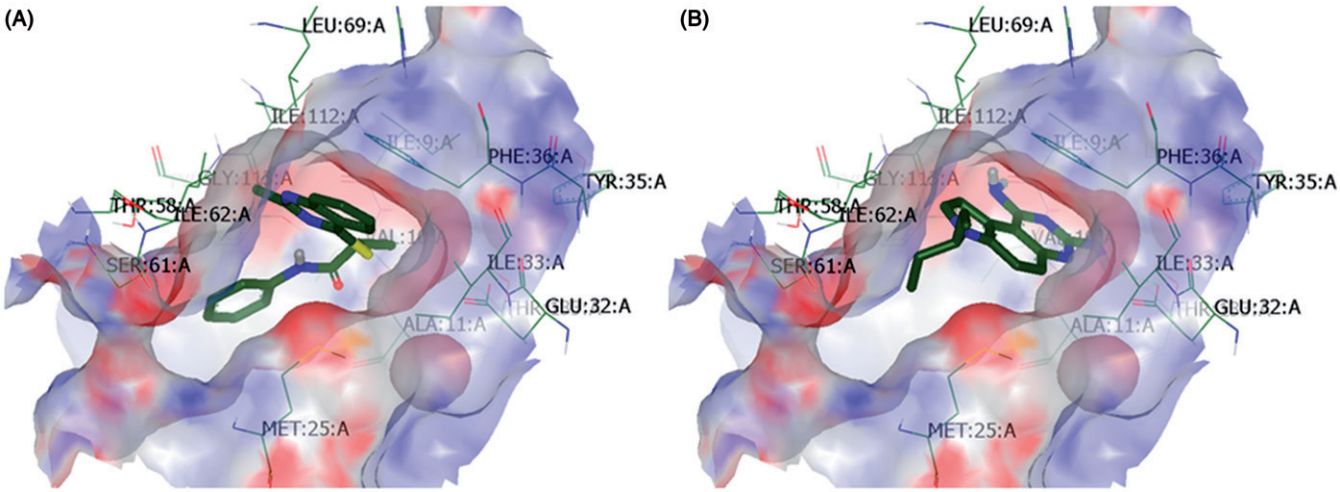


Figure 1. Interaction of 2-(2-methyl-quinazolin-4-ylthio)-*N*-phenylpropionamide (**4.6**) (**A**) and reference (7-(pentan-3-yl)-7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine) (**B**) into the DHFR binding site.

Table 1. The obtained scoring functions of the investigated compounds and (7-(pentan-3-yl)-7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine) into DHFR binding site.

Cmpd	Consensus Score	Shapegauss	PLP	Chemgauss2	Chemgauss3	Chemscore	OEChemscore	Screenscore	CGO	CGT	Zapbind
4.6	82	−378.765	−49.6334	−44.7116	−60.8656	−20.527	−33.3789	−116.047	−218.186	−0.43637	−22.6468
4.7	86	−444.152	−50.459	−49.3208	−60.7651	−20.6596	−34.9882	−121.381	−190.051	−0.32638	−26.0352
Reference	86	−308.194	−51.2794	−46.1962	−64.3525	−21.2196	−28.2021	−120.31	−351.28	−0.95831	−8.45465
4.3	92	−381.148	−49.2852	−46.3099	−59.078	−22.1063	−34.3712	−109.501	−208.71	−0.40294	−22.3413
4.5	97	−381.592	−47.7807	−45.2465	−68.0848	−18.4502	−34.1904	−120.006	−197.684	−0.42593	−22.6365
3.6	108	−383.317	−46.208	−44.6148	−62.5484	−17.9078	−30.9478	−102.223	−226.669	−0.49999	−19.3994
4.4	114	−365.394	−46.6807	−42.1459	−57.7325	−18.6133	−30.0201	−114.61	−209.083	−0.47499	−23.6368
3.4	137	−354.584	−43.5951	−40.4575	−57.537	−17.6772	−30.2581	−114.387	−227.122	−0.53644	−17.5635
4.2	137	−331.913	−44.9021	−37.9129	−46.694	−18.226	−27.8546	−109.7	−245.288	−0.56868	−19.1514
4.8	139	−360.515	−49.4909	−44.2391	−57.8334	−17.6949	−29.1051	−108.545	−235.902	−0.46207	−16.0701
2.6	145	−348.186	−49.9596	−40.3333	−53.3965	−20.8559	−32.1831	−120.94	−186.402	−0.44206	−17.7927
4.1	147	−345.766	−51.1229	−42.2129	−53.5865	−20.8751	−31.1928	−127.393	−195.133	−0.43923	−14.7374
3.5	157	−356.475	−48.9562	−44.038	−58.2667	−18.0081	−32.4152	−122.376	−200.883	−0.41885	−10.7625
2.5	197	−289.134	−41.4663	−35.6895	−52.4105	−15.6978	−25.8349	−111.403	−198.767	−0.45301	−19.2544
2.13	200	−259.853	−40.9669	−33.8891	−42.4025	−16.9804	−24.039	−96.9218	−272.537	−0.73395	−18.7643
3.9	215	−300.149	−38.0297	−36.7167	−42.1042	−13.8311	−27.2424	−95.5493	−240.888	−0.59124	−17.0203
2.17	220	−347.948	−40.6094	−41.0362	−58.406	−15.1547	−27.4	−93.372	−101.988	−0.21461	−22.1789
2.14	223	−299.186	−41.2863	−34.2701	−46.2202	−16.6175	−25.0826	−101.669	−195.767	−0.45753	−17.8969
2.15	225	−293.334	−40.2826	−33.639	−47.1476	−16.0805	−24.8425	−103.112	−197	−0.46266	−17.6947
4.9	234	−372.463	24.61172	−45.7289	−55.1096	−15.4709	−28.1221	−26.1088	−160.542	−0.35169	−20.3654
2.16	239	−286.71	−40.4509	−33.5012	−46.304	−15.0294	−24.6116	−102.597	−197.935	−0.46985	−17.4272
2.12	242	−264.66	−37.1178	−34.8203	−52.7886	−14.3724	−22.2174	−92.4593	−227.895	−0.70836	−13.4663
2.18	250	−282.787	−32.8939	−33.8284	−39.9133	−14.785	−−25.2986	−89.7601	−218.89	−0.49911	−19.1477
2.4	253	−282.489	−39.6817	−32.4522	−45.3545	−15.2888	−24.9071	−102.492	−189.837	−0.46683	−17.0605
2.9	256	−279.662	−37.329	−34.5317	−40.7148	−15.3446	−24.0937	−86.958	−212.897	−0.61779	−14.6992
3.2	276	−256.281	−37.0266	−30.4889	−39.6976	−13.9405	−21.1586	−94.9782	−225.681	−0.66529	−16.4408
2.7	281	−245.564	−33.1216	−29.8359	−35.8302	−14.2575	−22.9476	−82.0469	−226.679	−0.53643	−19.9704
2.11	282	−276.842	−38.6947	−31.4477	−42.7806	−14.258	−23.472	−96.1665	−180.632	−0.46416	−17.242
3.7	284	−286.732	−28.7227	−32.8977	−29.3642	−15.1515	−24.506	−64.3374	−218.546	−0.36965	−22.1967
2.10	288	−263.681	−32.4705	−31.9649	−37.644	−13.4627	−22.2012	−84.7462	−232.8	−0.59302	−15.9064
3.8	307	−307.998	−33.4153	−39.6585	−44.5068	−10.1369	−19.2077	−86.2238	−149.599	−0.37068	−16.8518
3.1	307	−238.767	−34.8311	−28.7247	−37.8476	−12.8009	−20.9541	−90.6225	−216.915	−0.66458	−15.8733
3.3	311	−268.889	−32.1917	−38.5509	−41.7444	−12.482	−22.906	−78.2142	−194.199	−0.5021	−7.9952
2.8	347	−231.165	−34.8882	−29.3037	−36.1957	−9.09227	−16.0344	−85.5844	−185.563	−0.56516	−13.4571
2.3	364	−260.234	−33.4146	−29.595	−42.376	−12.449	−21.6917	−82.2969	−99.7401	−0.28716	−14.1796
2.2	393	−196.074	−24.9307	−22.9715	−35.0098	−9.1122	−16.4791	−70.2144	−107.419	−0.29593	−16.2219

Table 2. Microorganisms' growth inhibition zones (in mm).

Compound	Conc. (μg/disk)	*Staphylococcus aureus*	*Enterococcus aeruginosa*	*Klebsiella pneumonia*	*Candida albicans*
2.1	100	15	11	11	6*
2.2	100	6	6	6	23
2.3	100	6	6	6	16
2.4	100	15	10	10	8
2.8	100	9	6	6	6
2.9	100	11	6	6	18
2.10	100	6	6	6	13
2.11	100	6	6	6	15
2.15	100	6	6	6	10
Gatifloxacin	5	30	–**	–	–
Gemifloxacin	5	30	–	–	–
Moxifloxacin	5	31	–	–	–
Ceftriaxone	30	33	–	25	–
Nystatin	100	–	–	–	21
Ampicillin	10	22	16	–	–

All statistical analysis of the results was carried out using the ''Biostatistica'' software ($p \leq 0.05$).
*6 mm – diameter paper disk.
**Not investigated.

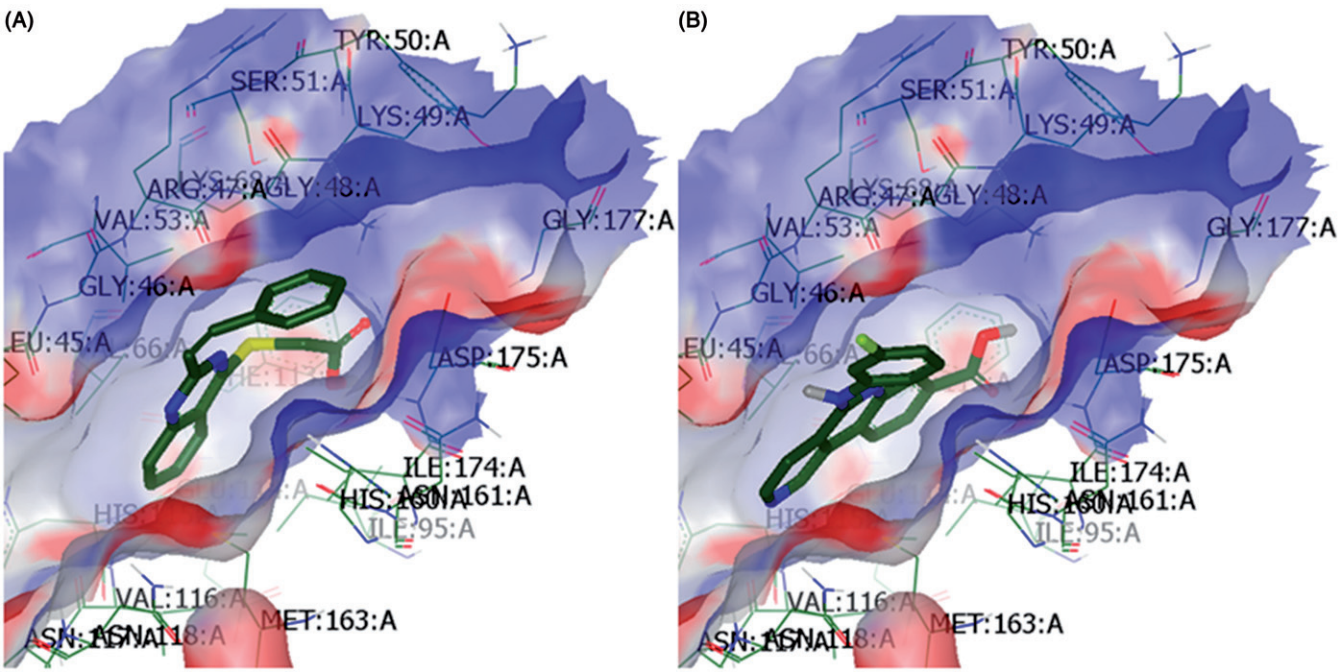


Figure 2. Interaction of 2-styryl-(quinazolin-4-ylthio)acetic acid (**3.7**) (**A**) and Silmitasertib (CX-4945) (**B**) into the binding site of protein kinase CK2.

a constitutively active serine/threonine kinase, that is involved in a variety of roles essential to the maintenance of cellular homeostasis. It regulates multiple processes that play important roles in the sensitivity of cancer to epidermal growth factor receptor targeting therapeutics, including PI3K-Akt-mTOR signaling, Hsp90 activity and inhibition of apoptosis[27]. Hence, we conducted docking studies of synthesized substances into the active site of protein kinase CK2 (3NSZ)[21]. Silmitasertib (CX-4945) was used as the reference (Figure 2)[29].

According to docking results, 11 substances had better Consensus Scores than Silmitasertib. Among them, the best two were 2-styryl-(quinazolin-4-ylthio)acetic acid (**3.7**) and *N*-(4-chlorophenyl)-2-(2-methyl-quinazolin-4-ylthio)acetamide (**4.9**) with Consensus Scores three times less than that of Silmitasertib, representing their anticancer potential (Figure 2, Table 3).

Thus, introduction of the amide residues again facilitates substances affinity. So, substances structures were sent to the National Cancer Institute (NCI) to be *in vitro* investigated for anticancer properties.

Preliminary in vitro anticancer testing. The 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 (Table 4).

Hence, 2-trifluoromethyl-(quinazolin-4-ylthio)acetic acid (**3.8**) was the most active among all, inhibiting growth of UO-31 of renal cancer to 17.01% and A498 to 54.81%. While it had promoted the growth of CNS cancer SF-268 line, leukemia CAKI-1 and SR lines, and ovarian cancer OVCAR-3. The best anticancer compound against leukemia CCRF-CEM cell line appeared to be 2-(3,4-dimethoxyphenyl)-3*H*-quinazoline-4-thione (**2.18**) with 18.75% of cancer line growth. 2-Benzyl-3*H*-quinazoline-4-thione (**2.5**) was practically ineffective against all cancer cell lines, except the light inhibition of UO-31 and A498 of renal cancer with 75.05 and 80.24%.

2-Trifluoromethyl-3*H*-quinazoline-4-thione (**2.8**) also showed light anticancer effect against ovarian IGROV1 to 71.97% and

Table 3. The obtained scoring functions of the investigated compounds and Silmitasertib into CK2 kinase binding site.

Cmpd	Consensus score	Shapegauss	PLP	Chemgauss2	Chemgauss3	Chemscore	OEChemscore	Screenscore	CGO	CGT	Zapbind
3.7	46	−465.545	−57.0443	−52.0457	−89.526	−22.8763	−38.2368	−146.183	−272.397	−0.5101	−17.8824
4.9	65	−447.211	−54.6976	−47.7201	−75.0627	−22.0093	−35.6513	−134.385	−263.625	−0.4875	−19.4444
3.6	100	−428.575	−50.8626	−46.2184	−84.1132	−19.661	−31.9269	−122.944	−281.953	−0.51936	−15.6018
4.4	111	−442.753	−56.5359	−51.9282	−79.1107	−24.8452	−38.2661	−155.028	−214.612	−0.38081	−12.7306
3.5	113	−423.283	−46.3807	−45.3975	−77.7719	−19.6203	−32.312	−119.811	−269.899	−0.50498	−17.9316
4.1	114	−435.422	−48.305	−47.4608	−66.9817	−19.5518	−35.4336	−118.385	−269.485	−0.45643	−21.7419
4.7	116	−452.331	−48.8969	−47.4851	−67.022	−19.8292	−33.0239	−115.513	−258.918	−0.39834	−22.7315
3.9	125	−407.418	−50.5678	−43.645	−78.0435	−19.8355	−30.0673	−125.964	−244.859	−0.47028	−18.7362
4.3	125	−438.655	−47.5173	−48.4867	−73.9937	−18.5955	−33.0863	−125.574	−238.485	−0.37643	−19.7773
3.4	131	−390.288	−52.0469	−46.6193	−70.9293	−21.6011	−33.7218	−113.403	−245.244	−0.45564	−19.777
4.6	137	−400.893	−48.0687	−43.0718	−70.9628	−18.3473	−31.2201	−115.011	−268.464	−0.46281	−23.5911
Silmitasertib	158	−424.616	−44.4712	−47.3726	−69.2785	−18.7694	−32.451	−118.674	−244.346	−0.37861	−19.8628
4.8	168	−421.062	−45.1238	−45.1304	−67.259	−18.014	−30.1503	−115.397	−267.213	−0.48533	−18.925
2.13	174	−379.789	−49.9054	−40.2089	−75.7726	−18.8896	−28.2063	−115.153	−230.579	−0.46066	−19.2743
2.7	177	−388.947	−46.1489	−40.0884	−71.9881	−19.3879	−31.2235	−112.757	−253.82	−0.48786	−17.8278
2.5	185	−379.177	−44.743	−40.5407	−70.1908	−19.7693	−31.3653	−111.778	−234.62	−0.51387	−18.2424
4.2	187	−398.286	−42.2567	−43.9281	−65.9785	−18.0202	−30.0671	−110.607	−289.325	−0.48154	−19.2192
2.14	200	−259.853	−40.9669	−33.8891	−42.4025	−16.9804	−24.039	−96.9218	−272.537	−0.73395	−18.7643
2.15	215	−359.284	−41.9247	−37.9216	−70.7715	−17.1314	−26.9806	−113.381	−247.599	−0.49254	−21.8732
2.17	225	−384.701	−39.4418	−44.3643	−68.6724	−11.178	−27.1451	−113.632	−273.045	−0.53468	−17.3226
2.16	226	−367.321	−49.5066	−39.297	−71.1119	−17.1016	−28.7606	−113.392	−223.74	−0.44982	−18.5348
4.5	243	−400.059	−43.1326	−44.8307	−51.2566	−19.4939	−30.6285	−114.266	−220.905	−0.37854	−12.3272
2.11	250	−374.852	−52.315	−38.1648	−69.8586	−17.1932	−28.4853	−109.82	−203.502	−0.40935	−17.2089
2.10	272	−359.428	−46.3294	−40.4289	−59.6429	−16.6666	−29.195	−95.8228	−226.098	−0.45694	−16.971
2.6	279	−348.078	−41.9709	−36.5865	−65.4811	−16.9829	−28.2629	−104.365	−205.341	−0.36445	−23.0116
2.9	287	−344.132	−42.0194	−35.3252	−61.3411	−16.4878	−25.7006	−105.813	−206.169	−0.41606	−20.1194
3.2	290	−331.338	−37.2939	−33.9678	−65.3822	−15.1261	−23.7235	−95.9809	−222.752	−0.48607	−20.6174
3.8	302	−330.22	−38.0283	−36.3433	−71.1171	−13.3303	−20.9295	−102.169	−227.065	−0.43846	−18.7044
2.4	306	−329.269	−42.851	−34.4855	−59.3032	−17.1053	−27.5289	−106.138	−186.669	−0.3445	−19.1691
3.3	313	−340.33	−39.5097	−37.3865	−58.6646	−15.0863	−27.1866	−114.204	−213.336	−0.3974	−3.82986
2.12	344	−319.216	−39.4209	−33.4432	−56.3152	−14.9212	−22.6947	−100.03	−171.279	−0.33309	−19.5219
3.1	348	−329.32	−41.1729	−37.3324	−56.1612	−15.4284	−26.6064	−105.983	−170.162	−0.30434	−16.2156
2.3	359	−284.88	−40.0202	−28.3876	−56.6993	−14.4478	−23.0104	−98.8193	−161.087	−0.34059	−18.7
2.18	360	−318.385	−33.8189	−32.8457	−49.0541	−12.8196	−22.0651	−88.9445	−190.158	−0.29216	−22.9582
2.8	366	−287.948	−40.3914	−30.1253	−58.4542	−13.0625	−21.1519	−97.3175	−171.226	−0.34107	−17.5733
2.2	400	−268.66	−35.0508	−26.1141	−51.8065	−12.9322	−21.0005	−85.8291	−149.89	−0.31695	−17.199
2.1	404	−268.407	−35.9859	−27.4171	−53.8422	−13.4993	−20.3355	−90.374	−124.304	−0.27049	−14.629

Table 4. Percentage of *in vitro* tumor cell lines growth with investigated substances in 10 μM.

	Compound/Growth percent			
Subpanel tumor cell lines	2.5	2.8	2.18	3.8
Leukemia				
CCRF-CEM	95.86	102.64	18.75	102.67
HL-60(TB)	98.58	97.17	97.02	108.99
K-562	nt	110.42	98.36	107.52
MOLT-4	105.14	117.38	90.50	116.67
RPMI-8226	nt	89.50	99.12	89.67
SR	95.49	108.57	91.75	150.08
Non-small cell lung				
A549/ATCC	104.38	95.59	96.01	96.80
EKVX	105.26	109.52	91.75	112.46
HOP-62	106.59	101.31	85.56	99.07
HOP-92	90.95	97.95	97.09	108.30
NCI-H226	102.59	113.93	114.06	101.31
NCI-H23	nt	96.66	99.19	89.87
NCI-H322M	103.03	107.47	114.93	96.83
NCI-H460	110.50	107.59	106.45	106.90
NCI-H522	92.96	88.20	87.26	92.36
Colon cancer				
COLO 205	131.96	101.98	111.56	113.19
HCC-2998	97.13	102.03	104.46	77.72
HCT-116	106.59	100.25	101.75	100.03
HCT-15	110.56	103.04	107.44	102.97
HT29	107.08	106.35	nt	104.49
KM12	117.43	114.67	106.26	113.99
SW-620	113.17	110.06	105.87	102.53
Renal cancer				
786-0	105.32	106.86	103.03	100.27
A498	80.24	84.51	96.78	54.81
ACHN	105.30	98.83	94.67	102.70
CAKI-1	87.12	73.23	87.51	160.10
RXF 393	120.11	108.49	105.43	112.15
SN12C	104.86	111.03	101.68	95.31
TK-10	109.08	111.71	143.35	102.22
105 to	nt	nt	nt	nt
Prostate cancer				
PC-3	96.85	103.10	89.33	98.05
DU-145	nt	146.23	123.87	147.82
Breast cancer				
MCF7	102.97	99.89	92.18	100.24
MDA-MB-231/ATCC	95.11	123.36	89.18	107.68
HS 578T	103.99	111.23	85.79	109.87
BT-549	98.85	106.65	100.39	94.03
T-47D	101.89	102.96	92.97	107.69

(*continued*)

Table 4. Continued

(*continued*)

Table 4. Continued

Subpanel tumor cell lines	Compound/Growth percent 2.5	2.8	2.18	3.8
MDA-MB-468	116.17	91.46	101.97	88.95
MDA-MB-435	107.35	111.08	95.21	107.42
NCl/ADR-RES	106.74	102.43	103.48	91.31
Ovarian cancer				
IGROV1	112.61	71.97	112.81	101.62
OVCAR-3	115.31	140.99	117.06	146.62
OVCAR-4	109.61	99.33	103.00	97.45
OVCAR-5	108.40	100.55	107.94	83.61
OVCAR-8	100.66	96.66	104.94	104.34
NCI/ADR-RES	106.74	102.43	103.48	91.31
SK-OV-3	113.26	102.10	100.75	101.22
Melanoma				
LOX IMVI	nt	97.13	109.57	97.75
MALME-3M	117.22	85.15	109.47	101.16
M14	97.74	101.45	111.92	100.37
SK-MEL-2	nt	102.81	125.84	104.18
SK-MEL-28	118.60	118.36	107.66	97.34
SK-MEL-5	100.44	99.46	87.39	109.72
UACC-257	107.03	94.13	99.95	99.36
UACC-62	105.96	121.79	84.18	99.53
MDA-MB-435	nt	111.08	95.21	107.42
CNS cancer				
SF-268	109.50	118.94	103.45	171.12
SF-295	98.53	123.25	108.42	109.27
SF-539	101.83	98.21	101.33	102.60
SNB-19	106.25	89.85	104.44	94.12
SNB-75	99.55	89.19	84.82	64.28
U251	101.57	95.33	101.21	102.51
Mean of data	104.38	103.49	99.96	103.00
Delta of data	29.33	31.52	81.21	85.99
Range of data	56.91	74.26	124.60	154.11

nt – Not tested.

renal cancer CAKI-1 to 73.23%, still promoting the growth of ovarian OVCAR-3 and prostate DU-145 cancer lines. Therefore, investigated substances have not undergone the predetermined threshold inhibition criteria to be progressed to the next 5-dose screen.

Structure–anticancer activity correlation. The best anticancer modification was synthesis of 2-(3,4-dimethoxyphenyl)-substituted 3*H*-quinazoline-4-thione, because introduction of carboxylic acid fragment and the halogen not only increased the anticancer properties, but procancer as well. Comparing docking results with *in vitro* one, substances **2.5**, **2.8** and **2.18** had low affinity to CK2 kinase. So, their anticancer activity mechanism should be of other inhibition pathway.

Conclusions

A number of novel 2-alkyl(aryl)-quinazolin-4-thiones, 2-R-(quinazolin-4-ylthio)carboxylic acids and amides were synthesized and characterized by their structure, *in silico* DHFR and CK2 affinity and *in vitro* biological activity. The antibacterial and antifungal screening against *S. aureus, E. faecalis*, *E. aerogenes, P. aeruginosa*, *E. coli*, *K. pneumonia* and *C. albicans* has found the most active unsubstituted (**2.1**), 2-methyl-(**2.2**) and 2-phenylquinazolin-4(3*H*)-thione (**2.4**). The modification of forth position with carboxylic acids and amides residues resulted in disappearance of any antimicrobial activity. The NCI anticancer study revealed 2-trifluoromethyl-(quinazolin-4-ylthio)acetic acid (**3.8**) and 2-(3,4-dimethoxyphenyl)-3*H*-quinazoline-4-thione (**2.18**) to have anticancer properties against renal cancer UO-31 and leukemia CCRF-CEM cell lines. Comparison of the *in silico* molecular docking for DHFR and CK2 kinase inhibition and *in vitro* biological activities speculatively had proven other antimicrobial and anticancer action mechanisms for synthesized substances. Thus, library of the antimicrobial and anticancer substances among 2-R-quinazolin-4(3*H*)-thione derivatives was enlarged to be used for future effective drug modifications. And, taking into the account their other non-investigated biological activities, studies of the novel synthesized compounds will be continued.

Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Supplementary material available online.
Supplementary Information