Synthesis of New 2-Thio-[1,2,4]triazolo[1,5-*c*]quinazoline Derivatives and Its Antimicrobial Activity

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A series of novel ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acids 2a—d and esters 3a—l were synthesized and evaluated for antimicrobial activity. Alkylation of potassium 2-thio-[1,2,4]triazolo[1,5-*c*]quinazoline 1 with halogenocarboxylic acids and its esters proceeded *S*-regioselectively. During acid catalyzed esterification of 2a—c, degradation of the pyrimidine ring was observed. The structures of the compounds were elucidated by FT-IR, ^{1}H- and ^{13}C-NMR, electron impact mass spectra (EI-MS) and LC-MS spectral data. Antimicrobial and antifungal activity of synthesized compounds was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Mycobacterium luteum*, *Candida albicans* and *Candida tenuis*. Acids 2a and 2c exhibited significant activity against *C. albicans*, which was additionally confirmed by the bioluminescence inhibition test and interrelated with their lipophilicity.

Key words 2-thio-[1,2,4]triazolo[1,5-*c*]quinazoline; antimicrobial; antifungal; bioluminescence; lipophilicity

Studies in the field of synthesis of new effective agents have become especially important in the recent years as a result of increasing spread of various infectious diseases. Triazoloquinazoline derivatives were of considerable interest due to their prominent biological properties. Recently, fifteen substituted [1,2,4]triazolo[4,3-*c*]quinazolines were tested for antibacterial and antifungal effects. As a result it was established that 9-chloro-5-morpholin-4-yl-3-(5-nitrothien-2-yl)-[1,2,4]triazolo[4,3-*c*]quinazoline **A** was the most effective compound, which has caused growth inhibition of *Bacillus subtilis*, *Staphylococcus aureus*, *Candida tropicalis* and *Rickettsia nigricans*.[1)] Two novel series of imidazo[2,1:5,1]-1,2,4-triazolo[4,3-*c*]quinazoline bearing 5-thioxo-1,2,4-triazoles and 4-oxothiazolidines were evaluated for antibacterial activity against representative Gram-positive and Gram-negative microorganisms. Among them tetracyclic derivative **B** was considered as a lead compound with promising activity displaying a higher potency than the reference standard ciprofloxacin.[2)] A certain antibacterial effect against *Bacillus subtilis* was manifested by *N*-aryl-(4-[1,2,4]triazolo[1,5-*c*]quinazolin-2-yl-thiazol-2-yl)acetamides (**C**).[3)] Furthermore, [1,2,4]triazolo[1,5-*c*]quinazolin-2-thiones **D** substituted at C(5) with (sulfo)alkyl group has been found to exhibit moderate antibacterial activity.[4—7)]

Thus, considering the fact of antimicrobial agents existence among 1,2,4-triazoloquinazolines, we were aimed to provide simple synthetic protocols of obtaining novel 2-thio-[1,2,4]triazolo[1,5-*c*]quinazoline derivatives in order to evaluate their antimicrobial and bioluminescence properties.

Experimental

Materials Potassium [1,2,4]triazolo[1,5-*c*]quinazoline-2-thiolate **1** and **2a** were synthesized according to the reported procedure.[8)] Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

Methods Melting points were determined in open capillary tubes in a Thiele's apparatus and were uncorrected. IR spectra (4000—600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer using a module for measuring attenuated total reflection (ATR). ^{1}H-NMR spectra (400, 500 MHz) were recorded on a Varian-Mercury 400 and Bruker Avance DRX-500 spectrometers with $SiMe_4$ as internal standard in DMSO-d_6 solution. ^{13}C-NMR spectra (125 MHz) were recorded on a Bruker Avance DRX-500 spectrometer with $SiMe_4$ as internal standard in DMSO-d_6 solution. LC-MS were recorded using chromatography/mass spectrometric system which consists of high-performed liquid chromatograph "Agilent 1100 Series" equipped with diode-matrix and mass-selective detector "Agilent LC/MSD SL" (atmospheric pressure chemical ionization (APCI)). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV. The purity of all obtained compounds was checked by ^{1}H-NMR and LC-MS.

General Procedure for the Synthesis of ([1,2,4]Triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic Acids (2a—d) The solution of halogenocarboxylic acid (11 mmol) in 5% aqueous KOH (12 ml) was added to the solution of **1** (10 mmol) in H_2O (15 ml). The mixture was stirred at the room temperature for 12 h. All insoluble materials were filtered off from the reaction mixture with addition of charcoal. The resulting solution was adjusted to pH 3—4 by adding 10% aqueous HCl solution. The precipitate was filtered, dried and recrystallized from the suitable solvent.

([1,2,4]Triazolo[1,5-*c*]quinazolin-2-ylthio)acetic Acid (2a) Compound **2a** was obtained as light yellow solid in 75.0% yield, mp 226—228 °C (lit.[8)] 226—228 °C) (2-propanol−H_2O). ^{1}H-NMR (400 MHz) δ: 4.05 (s, 2H, SCH_2), 7.73 (t, 1H, J=7.6 Hz, H-9), 7.84 (t, 1H, J=7.6 Hz, H-8), 7.98 (d, 1H, J=7.7 Hz, H-7), 8.40 (d, 1H, J=7.7 Hz, H-10), 9.30 (s, 1H, H-5), 11.43

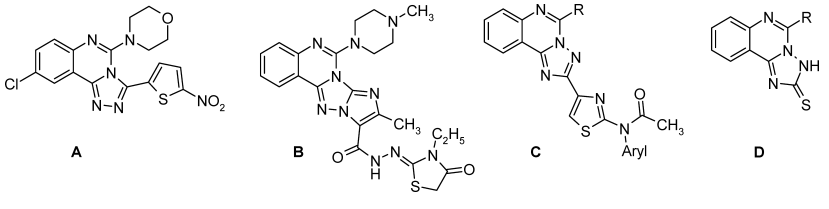

Fig. 1. Chemical Structures of the Selected Compounds with Antimicrobial Properties

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(br s, 1H, COOH). ^{13}C-NMR δ: 170.09 (COOH), 165.17 (C-2), 151.20 (C-10b), 142.79 (C-5), 138.49 (C-6a), 132.90 (C-8), 129.47 (C-7), 128.83 (C-9), 123.67 (C-10), 117.04 (C-10a), 34.02 (CH_2). IR (cm^{-1}): 2922, 1622, 1481, 1368, 1353, 1296, 1255, 1173, 1105, 1019, 968, 927, 908, 774, 715, 641. LC-MS *m/z*: 261 $[M+H]^+$, 263. EI-MS *m/z*: (I, %): 261 (8), 260 (12) $[M]^{+\cdot}$, 218 (15), 217 (35), 216 (100), 215 (77), 189 (7), 184 (10), 183 (4), 171 (17), 130 (5), 129 (17), 102 (10), 75 (5). *Anal.* Calcd for $C_{11}H_8N_4O_2S$: C, 50.76; H, 3.1; N, 21.53; S, 12.32. Found: C, 50.80; H, 3.12; N, 22.00; S, 12.55.

2-([1,2,4]Triazolo[1,5-*c*]quinazolin-2-ylthio)propionic Acid (2b) Compound **2b** was obtained as white solid in 73.2% yield, mp 182—184 °C (2-propanol−H_2O). ^{1}H-NMR* (400 MHz) δ: 1.66 (d, 3H, J=7.1 Hz, CH_3), 4.54 (q, 1H, J=7.3 Hz, SCH), 7.81 (t, 1H, J=7.6 Hz, H-9), 7.94 (t, 1H, J=7.8 Hz, H-8), 8.03 (d, 1H, J=7.8 Hz, H-7), 8.38 (d, 1H, J=7.8 Hz, H-10), 9.52 (s, 1H, H-5). ^{13}C-NMR δ: 173.07 (COOH), 165.40 (C-2), 151.15 (C-10b), 142.83 (C-5), 138.55 (C-6a), 132.95 (C-8), 129.53 (C-7), 128.89 (C-9), 123.73 (C-10), 117.14 (C-10a), 43.95 (CH), 19.02 (CH_3). IR (cm^{-1}): 2360, 1712, 1514, 1478, 1452, 1396, 1354, 1307, 1269, 1248, 1207, 899, 766, 715, 653. LC-MS *m/z*: 275 $[M+H]^+$, 276. EI-MS *m/z* (I, %): 275 (14.5) $[M+1]^{+\cdot}$, 231 (10.6), 230 (83.5), 229 (21.6), 215 (31.9), 202 (9.6), 198 (12.8), 197 (100.0), 171 (31.4), 170 (7.2), 146 (6.9), 145 (5.3), 130 (5.2), 129 (49.0), 103 (5.1), 102 (37.4), 88 (5.5),76 (7.8), 75 (12.1), 60 (5.0). *Anal.* Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.55; H, 3.67; N, 20.33; S, 11.68. Found: C, 51.80; H, 3.50; N, 21.07; S, 12.15.

3-([1,2,4]Triazolo[1,5-*c*]quinazolin-2-ylthio)propionic acid (2c) Compound **2c** was obtained as white solid in 68.9% yield, mp 176—178 °C (2-propanol−H_2O). ^{1}H-NMR* (400 MHz) δ: 2.79 (t, 2H, J=7.3 Hz, $SCH_2C\underline{H}_2$), 3.40 (t, 2H, J=7.3 Hz, SCH_2), 7.75 (t, 1H, J=7.6 Hz, H-9), 7.80 (t, 1H, J=7.6 Hz, H-8), 8.01 (d, 1H, J=7.7 Hz, H-7), 8.43 (d, 1H, J=7.7 Hz, H-10), 9.27 (s, 1H, H-5). ^{13}C-NMR δ: 173.21 (COOH), 165.46 (C-2), 151.27 (C-10b), 142.87 (C-5), 138.60 (C-6a), 132.90 (C-8), 129.47 (C-7), 128.87 (C-9), 123.75 (C-10), 117.17 (C-10a), 34.52 (SCH_2), 26.84 ($\underline{C}H_2$-COOH). IR (cm^{-1}): 2360, 1710, 1622, 1603, 1517, 1481, 1397, 1357, 1308, 1251, 1209, 1176, 900, 766, 716, 644. LC-MS *m/z*: 275 $[M+H]^+$, 276. *Anal.* Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.55; H, 3.67; N, 20.33; S, 11.68. Found: C, 51.98; H, 3.38; N, 21.10; S, 12.14.

5-([1,2,4]Triazolo[1,5-*c*]quinazolin-2-ylthio)pentanoic Acid (2d) Compound **2d** was obtained as light yellow solid in 56.6% yield, mp 242—244 °C (2-propanol−H_2O). ^{1}H-NMR* (400 MHz) δ: 1.80 (m, 6H, $SCH_2(C\underline{H}_2)_3$), 2.60 (t, 2H, J=7.3 Hz, SCH_2), 7.75 (t, 1H, J=7.6 Hz, H-9), 7.86 (t, 1H, J=7.6 Hz, H-8), 8.01 (d, 1H, J=7.7 Hz, H-7), 8.43 (d, 1H, J=7.7 Hz, H-10), 9.27 (s, 1H, H-5). IR (cm^{-1}): 2361, 1718, 1618, 1509, 1457, 1282, 1256, 1176, 903, 767, 717. LC-MS *m/z*: 303 $[M+H]^+$, 304. *Anal.* Calcd for $C_{14}H_{14}N_4O_2S$: C, 55.62; H, 4.67; N, 18.53; S, 10.60. Found: C, 54.80; H, 4.87; N, 18.35; S, 10.24.

* Signal of H in COOH group disappeared because of deuterium exchange.

General Procedure for the Synthesis of ([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthio)carboxylic Acids Esters (3a—l). Method A The appropriate halogenocarboxylic acid ester (6 mmol) was added to a suspension of **1** (6 mmol) in 2-propanol (15 ml). The resulting mixture was heated for 2 h. After cooling to room temperature five-fold excess of H_2O was added. The crystalline precipitate was filtered, dried and recrystallized from the suitable solvent.

Method B A solution of an appropriate ester (6 mmol) in 2-propanol (15 ml) was added to a solution of **1** (6 mmol) in H_2O (15 ml). The resulting mixture was refluxed for 2 h. Further work-up as in method A afforded proper esters.

([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthio)acetic Acid Methyl Ester (3a) Compound **3a** was obtained as white solid in 73.3% yield (method A), mp 154—156 °C (2-propanol−H_2O). ^{1}H-NMR (400 MHz) δ: 3.70 (s, 3H, OCH_3), 4.25 (s, 2H, SCH_2), 7.82 (t, 1H, J=7.8 Hz, H-9), 7.94 (t, 1H, J=7.8 Hz, H-8), 8.05 (d, 1H, J=8.0 Hz, H-7), 8.37 (d, 1H, J=8.0 Hz, H-10), 9.52 (s, 1H, H-5). IR (cm^{-1}): 2360, 1737, 1622, 1514, 1478, 1400, 1299, 1265, 1196, 1160, 996, 895, 773, 716. LC-MS *m/z*: 275 $[M+H]^+$, 276. *Anal.* Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.55; H, 3.67; N, 20.43; S, 11.69. Found: C, 53.50; H, 3.51; N, 21.43; S, 12.01.

([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthio)acetic Acid Ethyl Ester (3b) Compound **3b** was obtained as white solid in 76.4% yield (method B), mp 110—112 °C (2-propanol−H_2O). ^{1}H-NMR (400 MHz) δ: 1.21 (t, 3H, J=7.1 Hz, CH_3), 4.16 (q, 2H, J=7.0 Hz, $OC\underline{H}_2CH_3$), 4.23 (s, 2H, SCH_2), 7.82 (t, 1H, J=7.8 Hz, H-9), 7.94 (t, 1H, J=7.8 Hz, H-8), 8.05 (d, 1H, J=8.0 Hz, H-7), 8.37 (d, 1H, J=8.0, H-10), 9.52 (s, 1H, H-5). ^{13}C-NMR δ: 168.87 (C=O), 164.88 (C-2), 151.24 (C-10b), 142.85 (C-5), 138.55 (C-6a), 132.98 (C-8), 129.56 (C-7), 128.89 (C-9), 123.66 (C-10), 117.09 (C-10a), 61.17 (OCH_2), 33.70 (SCH_2), 14.53 (CH_3). IR (cm^{-1}): 2361, 1719, 1622, 1517, 1478, 1394, 1362, 1306, 1266, 1169, 1026, 901, 768, 715. LC-MS *m/z*: 289 $[M+H]^+$. EI-MS *m/z* (I, %): 289 (10.4), 288 (49.2) $[M]^{+\cdot}$, 243 (18.2), 242 (29.4), 217 (6.8), 216 (24.9), 215 (100.0), 214 (27.1), 202 (15.4), 187 (7.6), 171 (5.0), 130 (12.0), 129 (24.1), 102 (7.5). *Anal.* Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.2; N, 19.43; S, 11.12. Found: C, 53.80; H, 4.12; N, 20.08; S, 11.55.

2-([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Methyl Ester (3c) Compound **3c** was obtained as white solid in 52.8% yield (method A), mp 121—123 °C (2-propanol). ^{1}H-NMR (400 MHz) δ: 1.65 (d, 3H, J=7.2 Hz, $SCHC\underline{H}_3$), 3.70 (s, 3H, OCH_3), 4.62 (q, 1H, J=7.2 Hz, SCH), 7.81 (t, 1H, J=7.6 Hz, H-9), 7.94 (t, 1H, J=7.6 Hz, H-8), 8.04 (d, 1H, J=7.8 Hz, H-7), 8.37 (d, 1H, J=7.8 Hz, H-10), 9.53 (s, 1H, H-5). IR (cm^{-1}): 2360, 1732, 1623, 1514, 1476, 1453, 1392, 1303, 1262, 1173, 1088, 898, 772, 751, 716. LC-MS *m/z*: 289 $[M+H]^+$, 291. *Anal.* Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.2; N, 19.43; S, 11.12. Found: C, 53.23; H, 4.02; N, 20.01; S, 11.67.

3-([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Methyl Ester (3d) Compound **3d** was obtained as white solid in 80.9% yield (method A), mp 116—118 °C (2-propanol−H_2O). ^{1}H-NMR (400 MHz) δ: 2.89 (m, 2H, $C\underline{H}_2CO+H_2O$). 3.47 (t, 2H, J=7.3 Hz, SCH), 3.69 (s, 3H, OCH_3), 7.75 (t, 1H, J=7.8 Hz, H-9), 7.86 (t, 1H, J=7.8 Hz, H-8), 8.01 (d, 1H, J=8.0 Hz, H-7), 8.44 (d, 1H, J=8.0 Hz, H-10), 9.28 (s, 1H, H-5). IR (cm^{-1}): 2361, 1729, 1515, 1479, 1398, 1353, 1264, 1174, 901, 770, 717. LC-MS *m/z*: 289 $[M+H]^+$, 291. *Anal.* Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.2; N, 19.43; S, 11.12. Found: C, 53.85; H, 4.24; N, 19.95; S, 11.50.

4-([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthio)butyric Acid Ethyl Ester (3e) Compound **3e** was obtained as white solid in 11.1% yield (method A), mp 106—108 °C (2-propanol). ^{1}H-NMR (500 MHz) δ: 1.16 (t, 3H, J=6.8 Hz, CH_3), 2.05 (t, 2H, J=6.8 Hz, $SCH_2C\underline{H}_2$), 3.31 (m, 4H, $S\underline{C}H_2$, $S(CH_2)_2C\underline{H}_2CO+H_2O$), 4.06 (q, 2H, J=7.0 Hz, $OC\underline{H}_2CH_3$), 7.80 (t, 1H, J=7.2 Hz, H-9), 7.94 (t, 1H, J=7.2 Hz, H-8), 8.04 (d, 1H, J=7.8 Hz, H-7), 8.39 (d, 1H, J=7.8 Hz, H-10), 9.52 (s, 1H, H-5). IR (cm^{-1}): 2361, 1730, 1621, 1516, 1478, 1396, 1250, 1208, 1186, 1136, 1019, 901, 778, 717. LC-MS *m/z*: 317 $[M+H]^+$. EI-MS *m/z* (I, %): 316 (12.1) $[M]^{+\cdot}$, 271 (12.0), 230 (13.3), 229 (100.0), 216 (31.8), 215 (7.0), 203 (6.8), 202 (13.1), 130 (10.3), 129 (51.8), 115 (7.8), 103 (9.0), 102 (53.3), 90 (7.9), 88 (9.8), 87 (13.5). *Anal.* Calcd for $C_{15}H_{16}N_4O_2S$: C, 56.95; H, 5.10; N, 17.71; S, 10.13. Found: C, 57.14; H, 5.13; N, 16.95; S, 9.75.

5-([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthio)pentanoic Acid Methyl Ester (3f) Compound **3f** was obtained as white solid in 87.4% yield (method A), mp 96—98 °C (2-propanol−H_2O). ^{1}H-NMR (500 MHz) δ: 2.49 (m, 4H, $C\underline{H}_2C\underline{H}_2CO+H_2O$), 2.37 (t, 2H, J=7.3 Hz, $SCH_2C\underline{H}_2$), 3.29 (t, 2H, J=7.3 Hz, $S\underline{C}H_2$), 3.56 (s, 3H, OCH_3), 7.81 (t, 1H, J=7.3 Hz, H-9), 7.93 (t, 1H, J=7.3 Hz, H-8), 8.04 (d, 1H, J=8.1 Hz, H-7), 8.38 (d, 1H, J=7.8 Hz, H-10), 9.51 (s, 1H, H-5). IR (cm^{-1}): 2361, 1718, 1617, 1516, 1475, 1386, 1344, 1283, 1220, 1177, 977, 893, 769, 715, 642. LC-MS *m/z*: 317 $[M+H]^+$. *Anal.* Calcd for $C_{15}H_{16}N_4O_2S$: C, 56.95; H, 5.10; N, 17.71, S, 10.13. Found: C, 57.18; H, 5.23; N, 16.84, S, 9.86.

3-Phenyl-2-([1,2,4]triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Ethyl Ester (3g) Compound **3g** was obtained as white solid in 92.2% yield (method A), mp 128—130 °C (2-propanol−H_2O). ^{1}H-NMR (400 MHz) δ: 1.16 (t, 3H, J=7.3 Hz, CH_3), 3.32 (d, 2H, J=7.3 Hz, $SCHC\underline{H}_2CO$), 4.09 (q, 2H, ^2J=7.3 Hz, ^3J=1.8 Hz, $OC\underline{H}_2$), 4.73 (t, 1H, J=7.3 Hz, SCH), 7.20 (m, 1H, 4_{Ph}), 7.28 (m, 4H, H-2_{Ph},3_{Ph},5_{Ph},6_{Ph}), 7.88 (t, 1H, J=7.8 Hz, H-9), 7.78 (t, 1H, J=7.8 Hz, H-8), 8.03 (d, 1H, J=8.0 Hz, H-7), 8.45 (d, 1H, J=8.2 Hz, H-10), 9.30 (s, 1H, H-5). IR (cm^{-1}): 2972, 2926, 2352, 1721, 1594, 1515, 1477, 1453, 1369, 1299, 1263, 1239, 1175, 1030, 898, 862, 773, 747, 714, 698, 639. LC-MS *m/z*: 379 $[M+H]^+$, 381. EI-MS *m/z* (I, %): 379 (5.6) $[M]^{+\cdot}$, 204 (8.1), 203 (20.5), 202 (100.0), 129 (11.7), 102 (4.8), 91 (13.2). *Anal.* Calcd for $C_{20}H_{18}N_4O_2S$: C, 64.47; H, 4.79; N, 14.80; S, 8.47. Found: C, 64.10; H, 4.57; N, 15.31; S, 7.09.

3-(3-Methylphenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Ethyl Ester (3h) Compound **3h** was obtained as grey solid in 95.7% yield (method B), mp 67—69 °C (2-propanol−H_2O). ^{1}H-NMR (400 MHz) δ: 1.08 (t, 3H, J=7.2 Hz, $CH_2C\underline{H}_3$), 2.26 (s, 3H, CH_3), 3.26 (d, 2H, J=7.2 Hz, $SCHC\underline{H}_2$), 4.09 (q, 2H, J=7.2 Hz, $OC\underline{H}_2CH_3$), 4.70 (t, 1H, J=7.2 Hz, SCH), 7.05 (m, 3H, H-2_{Ph},4_{Ph},6_{Ph}), 7.18 (t, 1H, J=7.6 Hz, H-5_{Ph}), 7.82 (t, 1H, J=7.8 Hz, H-9), 7,94 (t, 1H, J=7.8 Hz, H-8), 8.04 (d, 1H, J=8.2 Hz, H-7), 8.37 (d, 1H, J=7.8 Hz, H-10), 9.52 (s, 1H, H-5). IR (cm^{-1}): 2362, 1736, 1623, 1515, 1477, 1391, 1304, 1270, 1172, 1158, 899, 773, 715, 699. LC-MS *m/z*: 393 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{20}N_4O_2S$: C, 64.27; H, 5.14; N, 14.27; S, 8.17. Found: C, 63.95; H, 4.72; N, 14.94; S,

8.09.

3-(3-Nitrophenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Methyl Ester (3i) Compound **3i** was obtained as light brown solid in 47.0% yield (method A), mp 80—82 °C (2-propanol). ^{1}H-NMR (400 MHz) δ: 3.43, 3.54, (dd, 2H, ^2J=13.9 Hz, ^3J=7.3 Hz, SCHC$\underline{H}_2$), 3.66 (s, 3H, OCH_3), 4.89 (t, 1H, J=7.6 Hz), 7.57 (d, 1H, J=7.6 Hz, H-6_{Ph}), 7.77 (d, 1H, J=7.6 Hz, H-4_{Ph}), 7.81 (t, 1H, J=7.8 Hz, H-9), 7.93 (t, 1H, J=7.8 Hz, H-8), 8.04 (d, 2H, J=7.8 Hz, H-7, H-5_{Ph}), 8.21 (s, 1H, H-2_{Ph}), 8.35 (d, 1H, J=7.8 Hz, H-10), 9.5 (s, 1H, H-5). IR (cm^{-1}): 1731, 1622, 1524, 1476, 1394, 1347, 1317, 1238, 1144, 954, 899, 815, 770, 735, 714. LC-MS *m/z*: 410 $[M+H]^+$, 411. *Anal.* Calcd for $C_{19}H_{15}N_4O_2S$: C, 55.74; H, 3.69; N, 17.11; S, 7.83. Found: C, 55.80; H, 3.48; N, 17.93; S, 7.99.

3-(4-Chlorophenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Methyl Ester (3j) Compound **3j** was obtained as white solid in 69.4% yield (method A), mp 112—114 °C (2-propanol). ^{1}H-NMR (400 MHz) δ: 3.31 (m, 2H, SCHC$\underline{H}_2$+H_2O), 3.65 (s, 3H, OCH_3), 4.78 (t, 1H, J=7.2 Hz, SCH), 7.33 (q, 4H, J=7.3 Hz, H-2_{Ph},3_{Ph},5_{Ph},6_{Ph}), 7.82 (t, 1H, J=7.6 Hz, H-9), 7.94 (t, 1H, J=7.6 Hz, H-8), 8.05 (d, 1H, J=7.8 Hz, H-7), 8.38 (d, 1H, J=7.8 Hz, H-10), 9.52 (s, 1H, H-5). IR (cm^{-1}): 2361, 1740, 1624, 1514, 1478, 1454, 1393, 1305, 1269, 1167, 1094, 1015, 900, 811, 774, 716. LC-MS *m/z*: 399 $[M+H]^+$, 401. *Anal.* Calcd for $C_{19}H_{15}ClN_4O_2S$: C, 57.21; H, 14.05; N, 8.02; S, 8.04. Found: C, 57.08; H, 13.94; N, 7.91; S, 8.15.

3-(2,4-Dichlorophenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Methyl Ester (3k) Compound **3k** was obtained as light yellow solid in 70.4% yield (method B), mp 104—106 °C (2-propanol). ^{1}H-NMR (400 MHz) δ: 3.34 (m, 2H, SCHC$\underline{H}_2$+H_2O), 3.67 (s, 3H, OCH_3), 4.87 (t, 1H, SCH), 7.32 (d, 1H, J=8.2 Hz, H-6_{Ph}), 7.44 (d, 1H, J=8.2 Hz, H-5_{Ph}), 7.53 (s, 1H, H-3_{Ph}), 7.82 (t, 1H, J=7.8 Hz, H-9), 7.94 (t, 1H, J=7.8 Hz, H-8), 8.05 (d, 1H, J=8.0 Hz, H-7), 8.34 (d, 1H, J=8.0 Hz, H-10), 9.50 (s, 1H, H-5). IR (cm^{-1}): 2361, 1737, 1619, 1512, 1473, 1401, 1356, 1353, 1229, 1148, 1052, 861, 845, 815, 769, 737, 714. LC-MS *m/z*: 435 $[M+H]^+$, 437. *Anal.* Calcd for $C_{19}H_{14}Cl_2N_4O_2S$: C, 52.67; H, 3.26; N, 12.93; S, 7.40. Found: C, 51.80; H, 3.54; N, 13.58; S, 7.05.

3-(4-Bromophenyl)-2-([1,2,4]triazolo[1,5-*c*]quinazoline-2-ylthio)propionic Acid Ethyl Ester (3l) Compound **3l** was obtained as white solid in 33.8% yield (method B), mp 78—80 °C (2-propanol). ^{1}H-NMR (400 MHz) δ: 1.08 (t, 3H, J=7.1 Hz, CH_3), 3.29 (m, 2H, SCHC$\underline{H}_2$+H_2O), 4.09 (m, 2H, OC$\underline{H}_2CH_3$), 4.75 (t, 1H, J=7.3 Hz, SCH), 7.26 (d, 2H, J=8.1 Hz, H-2_{Ph},6_{Ph}), 7.48 (d, 2H, J=8.1 Hz, H-3_{Ph},5_{Ph}), 7.83 (t, 1H, J=7.8 Hz, H-9), 7.95 (t, 1H, J=7.8 Hz, H-8), 8.06 (d, 1H, 8.1 Hz, H-7), 8.38 (d, 1H, J=7.8 Hz, H-10), 9.53 (s, 1H, H-5). IR (cm^{-1}): 2360, 1729, 1622, 1515, 1479, 1398, 1300, 1249, 1160, 1103, 1070, 1013, 900, 809, 317, 715, 692. LC-MS *m/z*: 457 $[M]^+$. EI-MS *m/z* (I, %): 412 (2.3) $[M+1-OC_2H_5]^+$, 411 (2.2), 385 (2.5), 215 (12.2), 209 (6.3), 204 (18.4), 203 (51.7), 202 (100.0), 171 (16.9), 169 (15.1), 134 (9.5), 129 (21.9), 102 (11.6). *Anal.* Calcd for $C_{20}H_{17}BrN_4O_2S$: C, 52.52; H, 3.75; N, 12.25; S, 7.01. Found: C, 51.74; H, 3.58; N, 12.84; S, 7.35.

4-([1,2,4]Triazolo[1,5-*c*]quinazoline-2-ylthiomethyl)benzoic Acid Methyl Ester (3m) Compound **3m** was obtained as white solid in 75.3% yield (method B), mp 144—146 °C (2-propanol). ^{1}H-NMR (400 MHz) δ: 3.83 (s, 3H, OCH_3), 4.60 (s, 2H, SCH_2), 7.69 (d, 2H, J=7.3 Hz, H-2_{Ph},6_{Ph}), 7.83 (t, 1H, J=7.8 Hz, H-9), 7.90 (m, 3H, H-8, H-3_{Ph},5_{Ph}), 8.05 (d, 1H, J=8.0 Hz, H-7), 8.40 (d, 1H, J=8.0 Hz, H-10), 9.53 (s, 1H, H-5). IR (cm^{-1}): 2360, 1714, 1621, 1513, 1478, 1396, 1355, 1266, 1184, 1105, 1016, 899, 766, 770, 740, 714. LC-MS *m/z*: 351 $[M+H]^+$, 352. EI-MS *m/z* (I, %): 351 (14.7), 350 (74.3) $[M]^{+\cdot}$, 318 (7.7), 317 (42.1), 258 (9.7), 215 (20.3), 201 (10.8), 188 (6.9), 173 (6.3), 171 (15.4), 150 (5.7), 149 (83.0), 146 (14.3), 130 (9.1), 129 (62.4), 122 (8.7), 121 (100), 118 (22.3), 104 (6.0), 103 (6.2), 102 (43.9), 91 (16.1), 90 (64.9), 89 (44.3), 88 (6.9), 78 (6.1), 77 (15.4), 76 (7.4), 75 (9.8). *Anal.* Calcd for $C_{18}H_{14}N_4O_2S$: C, 61.70; H, 4.03; N, 15.99; S, 9.15. Found: C, 61.40; H, 3.92; N, 16.18; S, 9.30.

Acid-Catalyzed Esterification of 2a—c. Method A To a solution of an appropriate carboxylic acid (**2a**—**c**, 5 mmol) in EtOH (10 ml) was added $SOCl_2$ (6 mmol) dropwise. The resulting mixture was refluxed for 3 h. After cooling to room temperature five-fold excess of H_2O was added and neutralized with $NaHCO_3$. The crystalline precipitate was filtered, dried and recrystallized from the suitable solvent.

Method B To a solution of an appropriate carboxylic acid (**2a**—**c**, 5 mmol) in EtOH (10 ml) was added H_2SO_4 (6 mmol). The resulting mixture was refluxed for 3 h. Further work-up as in method A afforded proper substances. The yield is stated for method A. Method B gives similar results with no more than 5%.

[5-(2-Aminophenyl)-4*H*-[1,2,4]-triazol-3-ylthio]acetic Acid Ethyl Ester (4a) Compound **4a** was obtained as brown solid in 46.8% yield (method A), mp 132—134 °C (2-propanol−H_2O). ^{1}H-NMR (500 MHz) δ: 1.17 (t, 3H, J=7.1 Hz, CH_3), 4.00 (s, 2H, SCH_2), 4.00 (q, 2H, J=7.3 Hz, OC$\underline{H}_2CH_3$), 6.58 (m, 2H, H-5_{Ph}+NH_{Ph}), 6.79 (d, 1H, J=7.6 Hz, H-3_{Ph}), 7.13 (m, 1H, H-4_{Ph}), 7.62 (m, 1H, H-6_{Ph}), 14.15 (br s, 1H, NH). IR (cm^{-1}): 3407, 3195, 3305, 3080, 2920, 2850, 1697, 1622, 1546, 1493, 1468, 1388, 1307, 1278, 1199, 1139, 1013, 858, 777, 751, 717, 673. LC-MS *m/z*: 279 $[M+H]^+$, 280. EI-MS *m/z* (I, %): 303 (11.7), 280 (7.3), 279 (20.9), 278 (100.0) $[M]^{+\cdot}$, 233 (13.2), 232 (20.0), 229 (8.6), 206 (20.6), 205 (43.0), 204 (21.1), 203 (29.5), 193 (5.8), 192 (46.2), 171 (13.5), 163 (5.5), 161 (9.0), 146 (9.0), 144 (10.4), 143 (6.4), 129 (21.4), 119 (40.3), 118 (74.8), 104 (9.5), 103 (9.8), 102 (9.3), 92 (16.8), 91 (28.2), 90 (6.8), 87 (5.5), 77 (12.1), 76 (7.2), 65 (12.7), 64 (6.2), 63 (5.6). *Anal.* Calcd for $C_{12}H_{14}N_4O_2S$: C, 51.78; H, 5.07; N, 20.11; S, 11.52. Found: C, 51.40; H, 4.99; N, 21.01; S, 10.96.

2-[5-(2-Aminophenyl)-4*H*-[1,2,4]-triazol-3-ylthio]propionic Acid Ethyl Ester (4b) Compound **4b** was obtained as brown solid in 60.0% yield (method B), oil. ^{1}H-NMR (500 MHz) δ: 1.12 (t, 3H, J=7.2 Hz, $OCH_2C\underline{H}_3$), 1.50 (d, 3H, J=7.2 Hz, SCHC$\underline{H}_3$), 4.08 (q, 2H, J=7.2 Hz, OC$\underline{H}_2CH_3$), 4.26 (q, 1H, J=7.2 Hz, SCH), 6.58 (m, 3H, H-5_{Ph}+NH_2), 6.78 (d, 1H, J=7.8 Hz, H-3_{Ph}), 7.12 (t, 1H, J=7.8 Hz, H-4_{Ph}), 7.67 (d, 1H, J=7.8 Hz, H-6_{Ph}), 14.30 (s, 1H, NH). IR (cm^{-1}): 3461, 3337, 3153, 2921, 2851, 1716, 1619, 1545, 1494, 1465, 1376, 1314, 1265, 1157, 1073, 1016, 980, 859, 745, 718, 673. LC-MS *m/z*: 293 $[M+H]^+$, 295. *Anal.* Calcd for $C_{13}H_{16}N_4O_2S$: C, 53.41; H, 5.52; N, 19.16; S, 10.97. Found: C, 53.35; H, 5.26; N, 20.03; S, 10.98.

5-(2-Aminophenyl)-4*H*-1,2,4-triazole-3-thiol (5) Compound **4c** was obtained as yellow solid in 57.7% yield (method A), mp 274—276 °C (2-propanol−H_2O). ^{1}H-NMR (500 MHz) δ: 6.17 (br s, 1H, NH_{Ph}), 6.54 (t, 1H, J=7.8 Hz, H-5_{Ph}), 6.73 (d, 1H, J=7.8 Hz, H-3_{Ph}), 7.06 (t, 1H, J=7.8 Hz, H-4_{Ph}), 7.65 (d, 1H, J=7.8 Hz, H-6_{Ph}), 13.44 (s, 1H, NH). IR (cm^{-1}): 3445, 3336, 2933, 2887, 2831, 2770, 1603, 1555, 1492, 1266, 1214, 1157, 1118, 1062, 1034, 966, 934, 773, 734, 697, 668. LC-MS *m/z*: 193 $[M+H]^+$, 195. *Anal.* Calcd for $C_8H_8N_4S$: C, 49.98; H, 4.19; N, 29.14; S, 16.68. Found: C, 49.92; H, 4.25; N, 29.08; S, 16.75.

Biological Activity Evaluation Methods Antimicrobial and antifungal activity of acids **2a**—**c** was investigated *via* the broth dilution method. Bacteria strains *Staphylococcus aureus* 209-p, *Escherichia coli* 675, *Pseudomonas aeruginosa* 165 and yeast *Candida albicans* 624 were used. Microorganisms were cultured on water aminopeptide solution (pH 7.2). The amount of bacteria in 1 ml of solvent was 2.5×10^5 colony forming unit (c.f.u.) after 18 h of treatment. The Sabouraud medium (pH 6.8, 500000 yeast bodies in 1 ml of solvent) was used for yeast growing. Antimicrobial activity was estimated by minimum inhibitory concentration (MIC)−the lowest concentration to completely inhibit bacterial growth of the compound shown in μg/ml. Compounds with MIC $\geq$40 μg/ml were considered to be inactive. Every experiment was repeated three times.

The investigation of antimicrobial and antifungal activity of esters **3h**—**l** was carried out with the stiff plate agar diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Mycobacterium luteum*, *Candida tenuis* and *Aspergillus niger*. The amount of microbial cells was 109 c.f.u./ml. Incubation period was 24 h at 35 °C for bacteria and 48—72 h at 28—30 °C for yeast. Antibiotics vancomicin, oxacillin, nystatin were used as standards. The bacterial cultures, standards, and obtained substances in 5 mg/ml concentration were streaked across grooves and then allowed to diffuse in the agar nutrient plate. The antimicrobial effect and degree of activity of the tested compounds were evaluated by measuring the zone diameters and the results were compared with well known drugs (Table 1). Every experiment was repeated three times.

Bioluminescence Inhibition Test The marine luminescent bacteria *Photobacterium leiognathi* Sh1, isolated from the Azov Sea, were used for the bioluminescence analysis.[9] Bacteria were cultivated on a nutrient environment containing (g/l): pepton, 5; yeast extract, 1.5; meat extract, 1.5; sodium chloride, 30; pH 7.4. In acute action test (inhibiting luminescence of bacteria) bacteria were diluted with the 3% sodium chloride solution up to concentration 10^5 cell/ml. The 5—50 μg/ml of the studied substances sus-

Table 1. Parameters of Antimicrobial Activity Evaluation

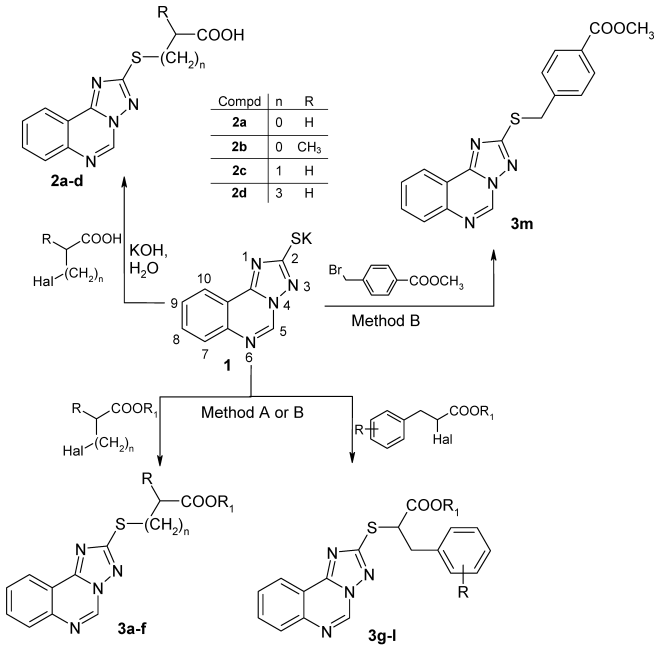
Inhibition zones (mm)	Degree of microorganisms activity
11—15	Not sensitive
16—25	Sensitive
>25	Highly sensitive

pended in DMSO were mixed with 1 ml of the diluted bacterial suspension. Vials were incubating for 10 min at 25 °C, then the intensity of bioluminescence was measured in % relatively to control tests that were performed without the studied compounds. In chronic action test (inhibiting growth and luminescence of bacteria) growth environment was added to the eventual breeding 1 : 50 and was incubated for 16—18 h at 30 °C, whereupon intensity of bioluminescence was measured in the same way as in the previous method. The bacterial luminescence was measured with Bioluminometer BLM-8801 (≪Science≫, Krasnoyarsk, Russia). The obtained results were used for creation of the luminescence intensity dependence curve from the control values and from the concentration of the studied substances.

Results

We were interested in introduction of carboxyalkylthio and alkoxycarbonylalkylthio groups onto the titled tricycle. This problem was solved using potassium 2-thio-[1,2,4]-triazolo[1,5-*c*]quinazoline **1** as starting material. The latter was synthesized according to the reported procedure[8] from the readily available 4-hydrazinoquinazoline and potassium ethylxanthogenate.

The alkylation of potassium [1,2,4]triazolo[1,5-*c*]quinazoline-2-thiolate **1** with halogenocarboxylic acids proceeded smoothly in the aqueous alkaline solution and neutralization with dilute HCl yielded the corresponding ([1,2,4]triazolo-[1,5-*c*]quinazolin-2-ylthio)carboxylic acids **2a**—**d**. Esters **3a**—**l** were obtained by refluxing the potassium salt **1** with proper halogenocarboxilic acid esters in alcoholic solution (method A) or aqueous alcoholic solution (method B) (Chart 1). Synthesized substances yielded oils, which were crystallized over a few days. In preparation of esters **3e** and **3l** we used bromo- instead of chlorocarboxylic acid esters. Thus, as bromine is less easily leaving group, the products yields decreased.



Compd	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l
n	0	0	0	1	2	3						
R	H	H	CH_3	H	H	H	H	3-CH_3	3-NO_2	4-Cl	2,4-Cl_2	4-Br
R1	CH_3	C_2H_5	CH_3	CH_3	C_2H_5	CH_3	C_2H_5	C_2H_5	CH_3	CH_3	CH_3	C_2H_5

Chart 1. Synthesis of ([1,2,4]Triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic Acids **2a**—**d** and Esters **3a**—**l**

Method A: 2-propanol. Method B: 2-propanol, water.

Discussion

Spectral data were used to evaluate the structures of obtained compounds.[8] Thus, IR spectra showed frequencies of certain molecular vibration in terms of single bonds of 1,2,4-triazoloquinazoline. Furthermore, the characteristic peak $\nu_{C=O}$ for carboxylic group of acids **2** was shown at 1718—1710 cm^{-1} and shifted to 1740—1714 cm^{-1} in spectra of the esters **3**. The NMR spectra contained signals of 1,2,4-triazoloquinazoline scaffold and proper substituents. The main characteristic ones in ^{1}H-NMR spectra **2**, **3** were singlet of H-5 (9.53—9.27 ppm) and doublet of H-10 (8.45—8.34 ppm), which were observed in low-field due to the strong deshielding caused by triazoloquinazoline ring. After deuterium exchange, the proton signal of COOH group disappeared in ^{1}H-NMR spectra of acids **2b**—**d**. The specific low field signals of C=O (173.21—168.87 ppm) were demonstrated in ^{13}C-NMR spectra of **2a**—**c** and **3b**. Alkylation of **1**, which is ambident nucleophile, could lead to both *S*- and *N*-derivatives, namely at N-3. The signal of C-2 (165.46—164.40 ppm) was the most prominent sign of the *S*-regioselectivity of this reaction. LC-MS confirmed the purity of obtained substances **2**, **3** observing appropriately protonated molecular ions $[M+H]^+$. The isotope patterns were quite different from each other, serving as fingerprints for some substances, which were identified accurately by following their ion-exchange separation into several fractions because of $^{35}Cl/^{37}Cl$ **3j**, **k** and $^{79}Br/^{81}Br$ **3l** isotopes.

In EI-MS the $[M]^{+\cdot}$ or $[M+1]^{+\cdot}$ peaks of the substances **2a**,**b** and **3b**, **e**, **g** appeared with low intensity. There was no molecular ion in **3l** spectrum, but $[M+1-OC_2H_5]^+$ ion with *m*/*z* 413 (2.3%) was present. The base peak of **3g**, **l** was fragment ion with *m*/*z* 202 (100%), showing one of the possible main molecule distraction directions by β-fragmentation forming a stable $[HetarylSH]^+$ ion.

Furthermore, we decided to elaborate an independent synthesis of esters **4a**, **b** by acid-catalyzed esterification from appropriate acids **2a**—**c**. However, in such case pyrimidine ring degradation was observed, and 5-(2-aminophenyl)-1,2,4-triazole derivatives were formed (Chart 2). Aside from that, the formation of product **5** by esterification of **2c** was complicated by β-elimination of the acrylate.

According to the IR spectroscopic data of the compounds **4a**, **b** and **5**, two characteristic absorption bands at 3445—3306 cm^{-1} were due to the appearing of the NH_2-group. The aromatic moiety of the **4a**, **b** and **5** protons in ^{1}H-NMR spectra appeared as two doublets and two triplets (7.65—6.54 ppm) each one integrating for one proton correspond-

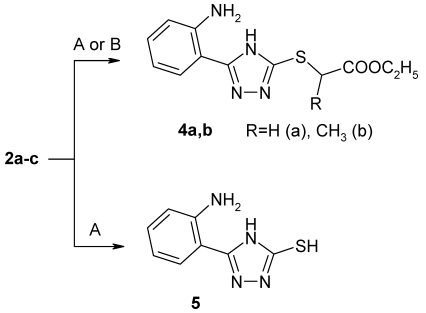


Chart 2. Acid-Catalyzed Esterification of **2a**—**c**

Method A: $SOCl_2$, EtOH, reflux for 3 h. Method B: H_2SO_4, EtOH, reflux for 3 h.

ingly. The NH proton of the triazole ring resonated as a broad singlet for **5** (13.44 ppm) and shifted to lowfield for **4a** (14.15 ppm) and **4b** (14.30 ppm). In EI-MS of **4a** there was a peak with *m*/*z* 192 (46.2%) demonstrating S–CH_2 bond breaking. The above-mentioned data were in accordance with literature reports for this heterocyclic system.[8)]

Antimicrobial Studies According to the results of microbiological screening, the synthesized acids **2a** and **2c** were found to possess antifungal activity against *Candida albicans*. This activity was lower than the action of miconazol, but was comparable with nystatin and even overcame itraconazole MICs. However, they were inactive towards Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria. Surprisingly, isomerization of **2b** side chain and its lengthening of **2d** led to the loss of antifungal activity (Table 2).

The antimicrobial effect of the tested esters **3h**—**l** was evaluated by measuring the inhibition zone diameters. Unfortunately, they demonstrated no inhibitory activity against both bacteria and yeast, which could be caused by protection of the carboxylic group (Table 3).

Bioluminescence Inhibition Test For additional estimations of biological action of the substances, the biocide activity of synthesized compounds was investigated *via* the bioluminescence (BL) inhibition test of the marine luminescent bacteria, which was commonly used for measurement of the chemical toxicity and ecotoxicity of water environment.[12)] The method allowed to expose different types of biological activity, related to influence on cellular membranes, its energy, synthetic apparatus, and fermentative systems.[13)] The results of BL test showed that the studied esters **3h**—**l** had no influence on bacterial BL as opposed to even stronger activity of acids **2a**—**c** in comparison with the reference in the acute action test (Table 4, Fig. 2).

In the chronic action test (inhibiting growth and bioluminescence of bacteria), acids **2a** and **2c** demonstrated biocide properties in concentrations of 0.25 mg/ml and 0.1 mg/ml, respectively, which is even lower than effective tetracycline concentration. The BL intensity was influenced by the action of esters **3h**—**l** and slightly increased with concentration of substances **3h**—**l** (Table 5, Fig. 3).

Moreover, it was noticeble that in the result of the BL test as shown in Fig. 3 and Table 5, change of BL intensity from added concentration of **2a** and **2c** was large. Values of **2a** BL (%) were 2000 (0.025 mg/ml) and 0.0 (0.1 mg/ml and 0.25 mg/ml). Furthermore, 285.7% of **2c** BL (0.025 mg/ml) was increased to 1428.6% (0.1 mg/ml) and then decreased to 0% (0.25 mg/ml). We considered these wide range of values with hormesis, a dose–response relationship phenomenon characterized by low-dose stimulation and high-dose inhibition, that had been frequently observed in properly designed studies and is broadly generalizable as being independent of chemical/physical agent or biological model. Among hormetic agents are numerous antibacterials, antivirals, antitumor, and antiangiogenesis agents, synthetic herbicides, peptides and allelopathy phenomenon.[14)] Thus, such results would enlarge data of antimicrobials hormesis and research methods in toxicology as well as fundamental insights in

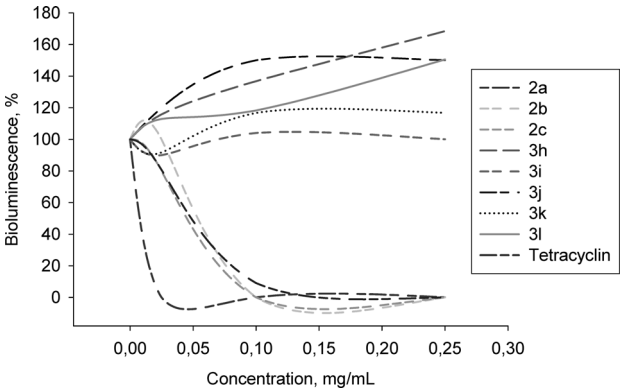


Fig. 2. Visualization of the BL in an Acute Action Test
Increasing curve represents induction of BL; decreasing, inhibition of BL.

Table 2. Minimum Inhibitory Concentration (MIC) of the Compounds **2a**—**d** (μg/ml)

Compound	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candida albicans*
2a	40.0	40.0	40.0	1.0
2b	40.0	40.0	40.0	40.0
2c	40.0	40.0	40.0	2.5
2d	40.0	40.0	40.0	40.0
Gentamicine[a)]	0.5	1.0	10.0	—
Itraconazole[a)]	—	—	—	16.0
Miconazole[a)]	—	—	—	0.125
Nystatin[a)]	—	—	—	2.0

a) According to the literature data.[10,11)]

Table 4. Values of BL in Acute Action Test (mg/ml)

Compound	Concentration (mg/ml)			
	0	0.025	0.1	0.25
2a	100.0	0.0	0.0	0.0
2b	100.0	100.0	0.0	0.0
2c	100.0	80.0	0.0	0.0
3h	100.0	115.8	136.8	168.4
3i	100.0	90.0	104.0	100.0
3j	100.0	120.0	150.0	150.0
3k	100.0	91.7	116.7	116.7
3l	100.0	112.9	118.3	150.5
Tetracyclin	100.0	80.7	9.1	0.0

Table 3. The Inhibitory Zones of the Compounds **3h**—**l** (mm)

Compound	Concentration (mg/ml)	*Escherichia coli*	*Staphylococcus aureus*	*Mycobacterium luteum*	*Candida tenuis*	*Aspergillus niger*
3h—**l**	5.0	—[a)]	—	—	—	—
Vancomicin	0.1	16	18	58	—	—
Nystatin	0.1	0	11	15	24	25
Oxacillin	0.1	0	21	—	—	—

a) Bacteria are resistant to the tested compounds.

Table 5. Values of BL in Chronic Action Test (mg/ml)

Compound	Concentration (mg/ml)			
	0	0.025	0.1	0.25
2a	100.0	2000.0	0.0	0.0
2b	100.0	83.3	333.3	0.0
2c	100.0	285.7	1428.6	0.0
3h	100.0	87.4	98.4	114.8
3i	100.0	50.0	20.0	20.0
3j	100.0	114.8	98.4	114.8
3k	100.0	150.0	266.7	466.7
3l	100.0	98.4	65.6	109.3
Tetracyclin	100.0	0.0	0.0	0.0

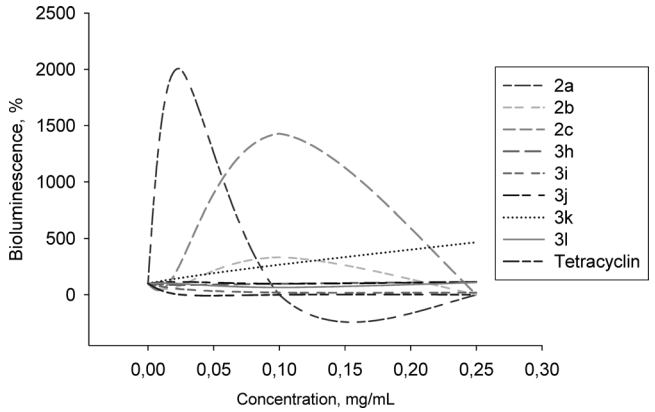


Fig. 3. Visualization of the BL in a Chronic Action Test

Increasing curve represents induction of BL; decreasing, inhibition of BL.

Table 6. Lipophilicity ot the Acids (**2a**—**c**)

Compound	EC_{50} (μg/ml)	log P	log D	MIC (μg/ml)
2a	8	1.71	−1.98	1.0
2b	54	2.06	−1.62	40.0
2c	46	1.88	−1.37	2.5

evolution biology.

Summing up the results of BL assays, substances **2a**—**c** inhibited bioluminescence of bacteria in the acute action test in smaller concentrations than in the chronic action test. Besides, on the base of above graph effective concentrations causing bioluminescence inhibition 50% (EC_{50}) in acute action test for the most active substances were obtained (Table 6).

Successful drug development requires not only optimization of specific and potent pharmacological activity at the target site but also efficient delivery to that site. Lipophilicity is well known as a prime physico-chemical descriptor of xenobiotics with relevance to their biological properties. It is considered to be one of the most significant reporters from the development of quantitative structure–activity relationship (QSARs) analyses.[15,16] Values of log P and log D were calculated with ACD/Log D software at pH=7.4.[17] It was stated that one should design drugs so that log P should be lower than 2.0.[15] Hydrophobic drugs tend to be more toxic because in general they are retained longer, have a wider distribution within the body, are somewhat less selective in their binding to proteins, and finally are often extensively metabolized. Hence it is advisable to make the drug as hydrophilic as possible while still retaining adequate binding affinity to the therapeutic protein target. As it is shown on the Table 5, lipophilicites of the investigated substances were of necessary rate as for neutral species (log P) so in the physiological pH of blood serum (log D). Moreover it was found that biocide BL activity and MICs of the acids **2a**—**c** decreased with the increasing of their lipophilicity.

Conclusions

We provided here preparative methods for the synthesis and evaluation of antimicrobial activity of new 2-thio-[1,2,4]triazolo[1,5-*c*]quinazoline derivatives. It was confirmed by spectral data that the alkylation of potassium salt **1** with halogenocarboxylic acids and its esters proceeded *S*-regioselectively with formation of appropriate substances. An attempt to obtain esters by the acid-catalyzed esterification of proper acids **2a**—**c** led to the formation of subsequent *o*-aminophenyl substituted triazoles. Among the obtained substances, acids **2a** and **2c** were found to possess strong antifungal activity towards *Candida albicans* in the most widely employed models for identification of antimicrobial activity. Also, there was a correlation between the lipophilicity and antimicrobial activity of **2a**—**c**. Moreover, this effect was confirmed by BL inhibition test. Besides, in the chronic action BL test substances **2a** and **2c** showed hormesis phenomenon. As the result we can say that synthesized ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acids **2a**—**c** are more active than esters **3a**—**l** when their antimicrobial activity is compared. It is worth mentioning that the relationship between antimicrobial activity, bioluminescence and lipophilicity of studied substances have been rarely discussed in literature, and would be important for further studies in the field of antimicrobial agents and QSAR analysis.

References and Notes

1) Jantova S., Ovadekova R., Letasiova S., Spirkova K., Stankovsky S., *Folia Microbiol.*, **50**, 90—94 (2005).
2) Magda N. N., Gineinah M. M., El-Bendary E. R., *Arch. Pharm.*, **336**, 560—566 (2003).
3) Likhate M. A., Fernandes P. S., *J. Indian Chem. Soc.*, **67**, 862—863 (1990).
4) Ibrahim S. S., Abdel-Halim A. M., Gabr Y., El-Edfawy S., Abdel-Rahman R. M., *J. Chem. Res.*, **5**, 1041—1063 (1997).
5) El-Sayed R., Wasfy A. A. F., Aly A. A., *J. Oil Soap Cosmetics*, **53**, 247—251 (2004).
6) El-Sayed R., Wasfy A. A. F., *J. Chin. Chem. Soc.*, **52**, 129—135 (2005).
7) El-Sayed R., Wasfy A. A. F., Aly A. A., *J. Heterocycl. Chem.*, **42**, 125—130 (2005).
8) Karpenko A. V., Kovalenko S. I., Shishkina S. V., Shishkin O. V., *Monatsh. Chem.*, **137**, 1543—1549 (2006).
9) Katsev A. M., Makemson J., Uchenye zapiski Tavricheskogo Natsionalnogo Universiteta im. V. I. Vernadskogo. Series "Biology, Chemistry," **19**, 111—116 (2006) (Russ. Ed.).
10) Dorrell L., Edwards A., *Sex. Transm. Infect.*, **78**, 308—309 (2002).
11) Barrow E. W., Valderas M. W., *Int. J. Antimicrob. Agents*, **27**, 178—180 (2006).
12) Girotti S., Ferri E., Fumo M., Maiolini E., *Anal. Chim. Acta*, **608**, 2—29 (2008).
13) Parvez S., Venkataraman C., Mukherji S., *Environ. Int.*, **32**, 265—268 (2006).
14) Calabrese E. J., Baldwi L. A., *Annu. Rev. Pharmacol. Toxicol.*, **4**, 175—197 (2003).
15) "Lipophilicity in Drug Action and Toxicology," ed. by Pliska V., Testa B., van de Waterbeemd H., VCH, Weinheim, 1996.
16) Cronin D., Mark T., *Curr. Comput.-Aided Drug Des.*, **2**, 405—413 (2006).
17) ACD/labs® 6.00, Advanced Chemistry Development Inc., 90 Adelaide Street West Toronto, Ontario, M5H 3V9, Canada.